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THE CEREBELLUM OF NECTURUS AND OTHER URODELE AMPHIBIA

C. JUDSON HERRICK

From the Anatomical Laboratory of the University of Chicago

THIRTY FIGURES

The cerebellum of lower urodeles has long been known to be in a greatly reduced condition; indeed, several authors have stated that in some of these forms it is entirely absent or represented merely by a dorsal commissural band of fibers. The subject obviously presents some features of general morphological interest; accordingly, I have examined the cerebellum in such American species of Urodela as are readily available, selecting *Necturus maculosus* (Raf.) and *Amblystoma tigrinum* (Green) for more detailed study. The observations on the development and adult structure of the latter type are reserved for a later report.

For nearly all of the material upon which this paper is based, including a very extensive series of microscopical preparations and a number of carefully made dissections, I am indebted to the kindness of Dr. Paul S. McKibben.

All of the Amphibia studied possess cerebellar tissue in typical relations and with essentially the same fibrous connections as in other vertebrates, save for the absence of the mammalian brachium pontis and its connections.

The embryological development of the cerebellum of mammals is known to begin as a thickening of the rhomboidal lip along the rostral border of the lateral recess of the fourth ventricle. In adult urodeles the recessus lateralis is very extensive and in the lower forms the configuration is very similar to that of very early embryonic stages of mammals in that the cerebellar tissue is present only in the walls of the wide lateral recesses, save for a commissural band of nerve fibers in the brain roof in front of them.

NECTURUS

The external form and the more important internal structures of the brain of *Necturus* have been well described in Kingsbury's excellent paper published in 1895. More detailed figures of the surface anatomy and the relations of some of the cranial nerves were published by McKibben in 1913.

It is difficult to fix the brain of *Necturus* for either macroscopic or microscopic examination without some distortion of its form, even when the brain is left *in situ* in the cranium during fixation. McKibben ('13, p. 155) has given instructions for reducing this distortion to a minimum. Figure 1 presents a dorsal view of a dissection of the cerebellar region and figures 2 and 3 a view from behind and very slightly from above of another specimen. Both of these brains were fixed *in situ* in Zenker's fluid in which the acetic acid was replaced by 10 per cent formalin before the dissections were completed and the chorioid plexuses removed. Figures 12 to 16 were drawn from cross sections of a specimen in which the distortion is very slight save for some dorso-ventral flattening. Figures 4 to 11 were drawn to illustrate the fiber tracts and the rather complicated technical procedure involved in their preparation has caused more distortion of the external form.

The recessus lateralis rhombencephali of *Necturus* is a wide expansion of the rostral end of the fourth ventricle, a part of which extends forward on each side of the midbrain as a blind anterior diverticulum. The 'auricular lobe' thus constituted is in a general way similar to that of selachian and some ganoid fishes. Figure 2 is drawn from a point of view which reveals the full extent of the lateral recesses and anterior diverticula. The walls of the anterior diverticulum are massive on all sides (figs. 4, 5, 6, 12, 13, 17). Part of its medial wall is fused with the lateral wall of the mesencephalon, but its anterior end is free (figs. 21, 22). The roof of each lateral recess (except the anterior diverticulum) is membranous and plexiform, this portion of the chorioid plexus of the fourth ventricle being evaginated and extending both lateralward and forward far beyond the limits of the underlying massive wall (figs. 14, 21), as Kingsbury has mentioned. That portion of

the lateral wall of the recess which borders the plexiform evagination is thin but massive. A few flattened neurones are found here, which appear to be greatly reduced Purkinje cells (figs. 17, 19). The massive walls of the anterior diverticulum are also relatively thin and undifferentiated.

At the rostral tip of the anterior diverticulum the superficial white layer is very thin and some cerebellar neurones are scattered through it, the stratum griseum thus reaching the external surface of the brain (fig. 22). This is a persistent vestige of a feature which is much more evident in urodele embryos.

The area acustico-lateralis forms the lateral wall of the fourth ventricle from about the level of the vagus roots forward nearly to the tip of the auricular lobe. The taenia ventriculi quarti borders its dorsal surface (figs. 1, 2, 16). It receives the VIII and lateral line VII and X roots and is separated by a distinct longitudinal ventricular sulcus from the underlying trigeminal area (*s.lat.*, figs. 1, 3, 16, 27). A short distance caudad of the V roots there is a shallow transverse ventricular sulcus which divides the area into anterior and posterior lobes. The anterior lobe (*l.l.l.*, figs. 1, 3, 10, 11, 13, 14, 15, 22) is flatter than the posterior lobe and does not extend forward quite to the rostral tip of the auricular lobe.

The area acustico-lateralis corresponds to the structure which students of fish brains have commonly called the tuberculum acusticum (including the lobus lineae lateralis); but this structure in both fishes and amphibians is more nearly comparable with the entire area acustica of mammals than with the tuberculum acusticum. The latter term, accordingly, should no longer be applied to brains of the Ichthyopsida. Norris, in his descriptions of *Amphiuma* ('08, p. 536) and *Siren* ('13, p. 283), has identified an area of neuropil which forms the most dorsal part of the stratum album in the region of the VII and VIII nerves with the lobus lineae lateralis of fishes. This area receives the most dorsal lateral line root of the facialis and was referred to by Kingsbury ('95, p. 187) as the dorsal island of alba.

The recessus lateralis is bounded rostrally by an obliquely transverse lamina of nervous material which we shall later recog-

nize as the body of the cerebellum and it extends backward nearly as far as the VII roots. Its lateral wall is formed chiefly by the anterior lobe of the area acustico-lateralis (figs. 1, 22, 23). The floor of the recessus lateralis is formed in part by the eminentia trigemini (*em.V*, figs. 1, 15,) and farther forward (figs. 2, 3, 13, 17, 27) by an eminence which is the direct forward extension of the eminentia trigemini. This I term the eminentia ventralis cerebelli (*em. cb. v.*). It is directly continuous in front of the recessus lateralis with the body of the cerebellum.

In *Necturus* the amount of cerebellar tissue is very small and it is poorly differentiated, though there is no uncertainty regarding its character. Cerebellar tissue is found on all sides of the anterior diverticulum and throughout the antero-medial wall of the lateral recess. The cerebellar tissue which forms the anterior wall of the lateral recess is not extensive; but since the chief mass of the cerebellum in higher Amphibia and Reptilia seems to be developed in this region, it will be termed the body of the cerebellum (*corpus cerebelli—c.cb.*, figs. 7, 8, 14, 15, 17 to 23, 27).

The body of the cerebellum is a bilateral structure whose two chief masses are connected across the mid-dorsal plane by the cerebellar commissure (with which are associated decussating fibers of the IV nerve and fibers of the mesencephalic root of the V nerve the whole forming the so-called *decussatio veli*). In gross preparations there is no evidence of any other massive tissue in the mid-dorsal plane of the cerebellum, but sections show (fig. 25) under the fiber bundles a few nuclei, in addition to those of the ependyma, which may be of nervous character.

The cerebellar commissure is bounded rostrally by the tectum mesencephali, whose roof at this point is very thin, though massive, thus forming a *frenulum veli medullaris anterioris*. This forms the roof of a dorsal dilation of the mesencephalic ventricle, which I term the recessus posterior mesencephali (*r.p.m.*, fig. 25). The frenulum contains a collection of cells of the nucleus mesencephalicus trigemini arranged in a thin layer. These are always present laterally of the meson, but in the medial plane they may be absent. Even in the latter case the roof membrane is not a simple epithelium, but contains two or three rows of nuclei.

Whether any of these and the few subependymal nuclei under the cerebellar commissure (fig. 25) are functional neurones has not been determined.

The neurones of the cerebellum of *Necturus*, like those of the other parts of the brain, are arranged as a deep stratum griseum bordering the ventricle. Both dendrites and neurites are in general directed outward into the stratum album. These neurones have not been exhaustively studied, but so far as observed they seem like greatly simplified Purkinje neurones. No cells are impregnated in our Golgi preparations which can be compared with the granules of more highly developed cerebella.

Some neurones of the floor of the recessus lateralis are seen in figure 17. This illustrates a transverse section through the lateral recess at a level between those of figures 6 and 7. The dendrites of these cells are directed partly downward into the underlying tractus spino-cerebellaris and partly medialward at the outer border of the stratum griseum. The former may engage terminals of the tractus spino-cerebellaris and also unmyelinated ascending fibres from the area acustico-lateralis. The latter spread out among fascicles of coarse heavily myelinated fibers which pass between this portion of the cerebellum and the medial raphé and may receive secondary trigemino-cerebellar fibers by way of those fascicles. The neurites of these neurones can be followed for only a very short distance, probably entering the tractus cerebello-tegmentalis and brachium conjunctivum.

The collection of neurones last described is continuous forward with both the medial and the lateral walls of the recessus lateralis. The dorsal border of the lateral wall is very thin, containing one to three layers of cells beside the ependyma. The few imperfect impregnations of these cells which I have seen indicate that they are greatly reduced Purkinje neurones (fig. 19).

Figures 18 and 19 illustrate two horizontal sections taken through the anterior diverticula of the lateral recesses. They are from different specimens but from approximately the same level, figure 18 showing the left side and figure 19 the right side. The cerebellar neurones in this region send their dendrites forward and downward, where they engage terminals of the tractus

spino-cerebellaris, tractus hypothalamo-cerebellaris, tractus tecto-cerebellaris, and perhaps other tracts. Their neurites are directed in part forward into the brachium conjunctivum, as illustrated, and partly downward and backward under the recessus lateralis in the tractus cerebello-tegmentalis. Figure 20 shows two smaller neurones from a point farther dorsal in the medial wall of the anterior diverticulum or corpus cerebelli.

The white matter of the cerebellum consists of a superficial neuropil and of certain well defined fiber tracts which are partly medullated. The neuropil (stratum moleculare) covers the entire external surface and also extends along the medial border of the anterior diverticulum of the recessus lateralis into the region of fusion between the diverticulum and the mesencephalon (figs. 4, 5, 23). The medullated fibers of the cerebellum accumulate chiefly in the area of fusion just referred to, i.e., along the external border of the ventricular grey, as in the higher Amphibia. The general plan of the fibrous connections of the cerebellum is not fundamentally different from that of the area acustico-lateralis which occupies a similar position in the rhomboidal lip farther caudad.

The fiber connections of this region, so far as revealed by the Weigert method, have accurately described by Kingsbury ('95, p. 174), whose description I have confirmed, though a thorough study of the arrangement of the neurones and unmedullated fibers would yield much additional information. Fibers of the VIII nerve and the lateral line roots of the VII and X nerves spread throughout the area acustico-lateralis, but the precise regions of distribution of these several roots have not been determined. Internal arcuate fibers pass into the medial raphé from the entire region, and there are two strong uncrossed medullated association bundles running between its rostral end and the parts of the oblongata farther caudad. One of these runs in the dorsal and one in the ventral part of the acustico-lateral area and they are termed by Kingsbury respectively 'tract a' and 'tract b' (figs. 10, 11, 21, 22, 23). Neither these tracts nor any root fibers of the VIII or lateral line nerves can be traced forward in Weigert preparations from the area acustico-lateralis into the cerebellum, though these fibers may take this course after the loss of their

medullary sheaths and other unmyelinated connections may occur.

The ascending spino-cerebellar system is well developed in urodeles, being larger in *Amblystoma* than in *Necturus*. There are two of these tracts, a dorsal and a ventral, which in *Necturus* enter the cerebellum medially and ventrally of the area acustico-lateralis, with which they do not come into relation.

The tractus spino-cerebellaris ventralis is the larger of these tracts. It ascends in company with the fibers of the tractus spino-tectalis system in the great fasciculus lateralis of the spinal cord and oblongata. In the upper levels of the oblongata this mixed bundle becomes imperfectly separable from the other fibers of the fasciculus lateralis, lying laterally of the large fasciculus bulbotectalis, or bulbar lemniscus (*lm.*, figs. 4 to 11 and 27).

At the level of the superficial origin of the V nerve the mixed bundle (fig. 11, *tr.sp.cb.v. + tr.sp.t.*) occupies the space between the lemniscus and the motor root of the V nerve, both components of the bundle being myelinated and at this level indistinguishably mingled. They continue forward along the lateral border of the bulbar lemniscus (figs. 7 to 10) to the level where the body of the cerebellum joins the eminentia ventralis cerebelli in front of the recessus lateralis (figs. 6, 27); here the mixed tract turns lateralward and divides into its two components.

The cerebellar component again divides, some of its fibers spreading throughout the adjacent cerebellar tissue, others ascending as a compact bundle to form most of the coarse fibered component of the commissura cerebelli. The tectal component of the mixed tract turns medialward (fig. 5) and joins the tractus thalamo-bulbaris (figs. 4, 5, *tr.th.b.*) The further course of these tracts through the mesencephalon I have not followed in *Necturus*; they probably distribute in the same way as in *Amblystoma*, as described below.

In the upper levels of the oblongata there is a distinct fascicle of myelinated fibers immediately ventral to the spinal V tract, whose course cannot be followed so clearly as the tract last described. Its fibers appear to cross those of the V roots at their superficial origin (fig. 10, *tr.sp.cb.d.*) and to enter the cerebellum

dorsally of the ventral spino-cerebellar tract last described. It is probably the tractus spino-cerebellaris dorsalis and has been so named in figures 6 to 10. Some of its fibers may enter the fine fibered component of the commissura cerebelli, though here, as in *Amblystoma*, most of them evidently end uncrossed. This tract is very distinct in *Amblystoma*, where it can be followed far backward through the oblongata.

From the entire extent of the area acustico-lateralis internal arcuate fibers descend along the boundary between the stratum griseum and the stratum album. Some of these cross in the raphé; others descend on the same side. From the caudal parts of the cerebellum similar internal arcuate fibers pass downward into the tegmentum and formatio reticularis. These are regarded as the fore-runners of the mammalian tractus cerebello-tegmentalis system. A part of this system of internal arcuate fibers from both the cerebellum and the anterior lobe of the acustico-lateral area is directed forward into the mesencephalon. These fibers constitute the brachium conjunctivum. They arise from the more ventral parts of the cerebellum, none being traced from the dorsal part of the corpus cerebelli.

The fibers of the brachium conjunctivum are chiefly unmedullated and pass inward, forward and downward along the lateral border of the stratum griseum toward their decussation in the floor of the midbrain (*br.conj.*, figs. 4, 18, 19, 24, 27). The further course of these fibers has not been traced, nor have the cells of the nucleus ruber been identified with certainty.

In fishes there is a very important connection between the hypothalamus and the cerebellum, the tractus lobo-cerebellaris, or better, the tractus mammillo-cerebellaris. A similar connection appears to be present in *Necturus*, though the details have not been fully worked out. The dorsal part of the hypothalamus appears to be the chief efferent center of that region. Fibers of the mammillo-peduncular system arise from its dorsal and lateral surfaces and turn mesad and caudad into the tuberculum posterius. Here part of its fibers decussate in the ventral commissure and both direct and crossed portions pass backward into the tegmentum. These fibers are unmedullated and in Golgi prepara-

tions often appear coarsely varicose and thus distinguishable from others with which they are mingled. Many of them pass at once to the lateral surface of the tegmentum, reaching the surface at about the point of exit of the III nerve. Here they turn caudad and dorsad mingled with terminals of the superficial thalamopeduncular tract. Having reached the caudal border of the tectum, they terminate with widely branched varicose arborizations among the dendrites of the cerebellar neurones (fig. 19, *tr.m.cb.*). This tract is nowhere condensed into a compact fascicle, but seems rather a special portion of a diffuse hypothalamo-tegmental connection.

The cerebellar commissure crosses immediately caudad of the recessus posterior mesencephali and its fibers are joined near the medial plane by tracts of very coarse medullated fibers from the tectum which enter it from the dorsal and lateral borders of the recessus posterior. These fibers probably include the tractus tecto-cerebellaris and also a part of the mesencephalic V root.

The decussatio veli consists of separate fascicles of unmedullated and fine and coarse medullated fibers. The unmedullated fibers cross the mid-plane farther caudad than the others (*com.cb.l.*, figs. 5, 6, 17, 18, 21, 22, 23, 25, 26, 27) and laterally pass into the stratum moleculare of the walls of the lateral recess. Their cellular connections are unknown. In the medial plane the medullated fibers of the decussatio veli form a compact fascicle with the coarser fibers farther rostrad. The coarse fibered portion is composed partly of fibers of the mesencephalic V root (with which fibers of the IV nerve are mingled), whose relations are considered more in detail below, and partly of fibers of the tractus spino-cerebellaris ventralis. The finer medullated fibers lie adjacent to the unmedullated component and laterally spread out chiefly in the stratum griseum of the body of the cerebellum. The fine fibered medullated component is derived from the cerebellar grey which borders the recessus lateralis on all sides, particularly medially and ventrally. These fibers swing downward and slightly forward from their crossing in the cerebellar commissure to enter the body of the cerebellum, where some of them end. At the junction of this structure with the midbrain the remainder of these

fibers curve downward and backward, here coming into relation with the coarse fibers of the mesencephalic V root, and then spreading out in the floor and posterior walls of the lateral recess (*com.cb.*, figs. 5 to 8, 17, 27). None of these fibers leave the substance of the cerebellum, so far as revealed by Weigert preparations.

The determination of the exact relations of the root fibers of the trigeminus to the cerebellum is very difficult, and I have not as precise information on this point in *Necturus* as in larval *Amblystoma*. In the latter type I have found that practically all fibers of the sensory V root bifurcate immediately upon entering the brain, their ascending branches extending forward to the rostral end of the auricular lobe. Probably the same holds for *Necturus*, but there is no evidence that any of these fibers reach the cerebellum in *Necturus*.

The connections of the mesencephalic root of the V nerve in *Necturus* have been described by Osborn ('88), Kingsbury ('95), Johnston ('05) and Norris ('13). The two authors first mentioned have commented upon the fact that the mesencephalic V root is accompanied by a tract of coarse medullated fibers which can be followed caudad from the level of the superficial origin of the V nerve as far as the roots of the VII and VIII nerves, lying dorso-medially of the spinal V tract. Norris ('13, p. 269) describes in *Siren* a portion of the mesencephalic V root which descends from the superficial origin of the root "as far posteriorly as the level of the root of the seventh nerve." I find in *Amblystoma* that these coarse descending fibers arise by the bifurcation of the mesencephalic root fibers and end among the cells of the motor VII nucleus. The details of this connection will be described elsewhere. A similar condition doubtless prevails in *Necturus*, and these are probably the fibers referred to by Osborn and Kingsbury.

The mesencephalic V fibers are, moreover, mingled with those of a quite independent system of coarse medullated fibers. In *Amblystoma*, where I have studied this tract more carefully, its fibers have been followed forward into the post-optic commissure. This makes it probable that it is a crossed descending tract from the thalamus or midbrain and it is accordingly here termed provisionally *tractus thalamo-bulbaris*.

Reading forward in transverse sections, the tractus thalamo-bulbaris of *Necturus* is small and compact just caudad of the superficial origin of the V roots, lying dorso-laterally of the lemniscus and close to the ventricular grey. At the level of the V roots, this tract is crossed by the motor V root and is joined by the fibers of the mesencephalic V root, some of which are coarser than any of these (fig. 11, *tr.th.b. + mes.V.*). Rostrally of the V roots, the mixed system forms a series of small fascicles of coarse fibers bordering the ventricular grey and extending lateralward from a point dorsally of the lemniscus to the shallow sulcus which marks the medial boundary of the area acustico-lateralis (fig. 10). Still farther forward under the recessus lateralis the lemniscus turns lateralward and these coarse fibered fascicles lie dorsally of it (fig. 9), but separated from it by masses of heavily medullated fibers. In front of the recessus lateralis there is another rearrangement (fig. 5). Most of the trigeminal fibers separate dorsally, some to enter the commissura cerebelli, others to ascend into the tectum mesencephali at a higher level than any other fibers now under consideration. The remaining fibers, chiefly belonging to the tractus thalamo-bulbaris, are immediately joined by coarse fibers of the tractus spino-tectalis, as already described (fig. 4, *tr.sp.t. + th.b.*), and the mixed bundle as thus reconstituted ascends into the mesencephalon, lying deeper than the lemniscus and parallel with it. Its further course has not been studied in *Necturus*.

The fibers of the mesencephalic V root which join the commissura veli for the most part separate from it again to enter the tectum mesencephali of the same side, chiefly for distribution to the roof of the recessus posterior mesencephali, though some of them doubtless cross to the opposite side in the commissure.

From the preceding account it appears that the fibers of the mesencephalic root of the V nerve are mingled with two other coarse fibered medullated tracts, the tractus thalamo-bulbaris and the tractus spino-tectalis, and the analysis of this complex is very difficult. I have found no evidence that any fibers of the V roots terminate in the cerebellum. Those which pass through the cerebellum, including those which cross in the decussatio veli,

pass very close to the caudal end of the mesencephalic V nucleus and all of them may easily reach these cells. Sections stained for medullated fibers only are quite inadequate to resolve this point, for the cells referred to lie so close to the cerebellar commissure that fibers destined to reach these cells from the commissural tract might lose their medullary sheaths before leaving the commissural tract.

Hirsch-Tabor ('08, p. 727) and later Bindewald ('11) describe in *Proteus* a 'commissura intertrigemina' which is regarded by the latter author in *Proteus*, *Hypogeophis* and *Cryptobranchus* as a true commissure between the sensory trigeminal nuclei of the oblongata. This seems to include the entire cerebellar commissure of the present account, which I have found to be a much more complex structure in *Necturus*, *Amphiuma* and *Cryptobranchus*. Bindewald recognizes other elements than the trigeminal fibers in *Cryptobranchus* and to the present writer it seems probable that a renewed examination of the brain of *Proteus* will reveal other components of the commissure there also. Some fibers of my tractus spino-cerebellaris ventralis et dorsalis (especially the more dorsal system) probably come from the primary sensory trigeminal nucleus of the same side and are to be regarded as secondary trigemino-cerebellar fibers. Some of these fibers enter the cerebellar commissure, but even these fibers could not properly be described as a 'commissura intertrigemina,' for their terminus is probably the cerebellum rather than the trigeminal nucleus of the opposite side. The same conditions probably prevail in *Proteus*, for one of Hirsch-Tabor's figures ('08, p. 726, fig. 2) shows that this animal possesses grey matter in the wall of the anterior diverticulum of the recessus lateralis which corresponds in position and other relations so far as shown to the corpus cerebelli of my description. In larval *Amblystoma* I find a large sensory root of the trigeminus which ascends to end in the eminencia trigemini, and some fibers of this tract continue forward as a separate bundle accompanying the tractus spino-cerebellaris to the rostral end of the auricular lobe.

AMPHIUMA

I have examined the brain of a small specimen of *Amphiuma* means 42 cm. long, cut into horizontal sections and stained with iron hematoxylin and acid fuchsin, and I find the relations here essentially as in *Necturus*, though with some modifications in detail.

The blind anterior diverticulum of the lateral recess of *Amphiuma* extends farther forward than in *Necturus* (cf. figs. 29 and 21) and its roof is wholly membranous instead of massive (fig. 28). The plexiform lateral evagination from the recessus is much more extensive than in *Necturus* and extends forward almost to the posterior pole of the cerebral hemisphere. At the ventral part of the rostral end of the auricular lobe the central grey layer reaches the surface and the stratum album (moleculare) is there entirely wanting. This area of superficial grey attains its maximum development immediately ventrally of the level shown in figure 30 and is more extensive than in *Necturus*.

As in *Necturus*, there is practically no cerebellar tissue developed in the medial plane except the dorsal commissure. The body of the cerebellum is thin and forms the entire rostral wall of the recessus lateralis and medial wall of its anterior diverticulum. The entire lateral wall of this diverticulum (so far as it is massive) is formed by the anterior lobe of the area acustico-lateralis, which is separated by an endymal sulcus from the posterior lobe (fig. 30).

The differences between these relations and those described above for *Necturus* are for the most part modifications in the direction of the higher urodeles, as illustrated by *Amblystoma*, though the cerebellum itself is no larger in *Amphiuma* than in *Necturus*. The resemblance to *Amblystoma* is seen particularly in the membranous roof of the entire recessus lateralis and in the greater concentration of cerebellar tissue in the body. The dorsal portion of the lateral wall of the anterior diverticulum of the lateral recess, which in *Necturus* is thin but massive, in *Amphiuma* is membranous, and the taenia ventriculi quarti is attached directly to the area acustico-lateralis in this region as in *Amblystoma*.

Study of the medullated fiber tracts of the brain of *Amphiuma* confirms the description given above for *Necturus* in almost every point. Both dorsal and ventral spino-cerebellar tracts are present. The system of internal arcuate fibers from the cerebellum and area acustico-lateralis (tr. cerebello-tegmentalis) is well developed, most of these fibers descending to decussate in the same transverse plane as their cells of origin. But few medullated fibers of this system are directed forward to decussate under the mesencephalon. That is, the brachium conjunctivum is either unmedullated or very feebly developed.

The decussatio veli lies relatively farther ventrally than in *Necturus*. The unmedullated and fine fibered medullated components of the cerebellar commissure are similarly related in the two species. The coarse medullated fibers also seem to be similarly related to the mesencephalic V root and the tractus spino-cerebellaris, the former fibers greatly predominating. In *Necturus* the relation of these coarse fibers of the commissure to the tectum mesencephali is obscured by their mingling with those of the decussating IV nerve; but in *Amphiuma* the reduction of the IV nerve to a small vestige comprising less than 10 medullated fibers renders a clearer analysis possible. Here it can be easily seen that by far the greater number of these coarse commissural fibers after their decussation turn forward to enter the tectum for distribution to the large cells of the mesencephalic V nucleus. One small fascicle of such fibers ascends directly dorsally from their decussation near the medial plane to reach the cluster of mesencephalic V cells lying in the roof of the recessus posterior mesencephali. Few, if any, root fibers of the trigeminus in *Amphiuma* terminate in the cerebellum; they traverse this structure to reach their cells of origin in the tectum mesencephali.

CRYPTOBRANCHUS

In *Cryptobranchus alleghaniensis* the cerebellum is arranged essentially as in *Necturus*, but the organ as a whole is better developed. The recessus posterior mesencephali is very evident, while the recessus lateralis rhombencephali is less extensive than in *Necturus*. The latter does not extend forward as an anterior diverticulum beyond the transverse level of the decussatio veli and its roof is wholly membranous. The body of the cerebellum is more massive than in *Necturus* and a thin column of cells, in addition to the ependyma, connects the stratum griseum of the two sides across the roof in connection with the commissura cerebelli. The anterior lobe of the area acustico-lateralis is separated dorsally from the posterior lobe by a deep sulcus. It extends forward to the lateral angle of the recessus lateralis, where it fuses broadly with the body of the cerebellum. The fiber tracts have not been studied in detail, but so far as observed, they conform to the description already given for *Necturus*.

The cerebellum of *Cryptobranchus* occupies an intermediate position between those of *Amphiuma* and *Amblystoma*.

SUMMARY

The cerebellum of mammals is known to develop from the rhomboidal lip bordering the lateral recesses of the fourth ventricle. The early embryonic stages of the human cerebellum resemble rather closely the adult condition of *Necturus* save for the absence in the latter case of the pons flexure.

In *Necturus* each lateral recess is continued forward into a blind anterior diverticulum whose walls are massive on all sides. Cerebellar tissue is present in the antero-medial wall and the floor of the lateral recess and in the walls of its anterior diverticulum in the auricular lobe. The postero-lateral wall of the lateral recess is formed by a thickening, the anterior lobe of the area acustico-lateralis, intermediate in structure between the cerebellum and the posterior or primary lobe of the area acustico-lateralis.

The thin plate of cerebellar tissue in the antero-medial wall of the lateral recess of each side is called the body of the cerebellum

(corpus cerebelli), because it seems to be the direct precursor of the chief cerebellar mass of higher amphibians and reptiles. The cerebellar tissue in the floor of the lateral recess (eminentia ventralis cerebelli) gives rise to the greater part of the feebly developed brachium conjunctivum and is therefore probably the primordium of the roof nuclei and the nucleus dentatus of the mammalian cerebellum.

The corpora cerebelli of the two sides are connected dorsally by a strong commissural system. In *Necturus* and *Amphiuma* the grey matter of the cerebellum does not reach the mid-dorsal plane except for a few cells of doubtful significance associated with the cerebellar commissure. In *Cryptobranchus* there is a thin bridge of grey matter extending across the mid-dorsal plane, which in higher urodeles like *Amblystoma* becomes massive.

The fiber connections of the cerebellum of *Necturus* are essentially similar to those of the area acustico-lateralis farther back in the rhomboidal lip, save for the presence of the dorsal cerebellar commissure and the absence of demonstrated root fibers of the cranial nerves. Fibers of the VIII and lateral line nerves terminate freely throughout the area acustico-lateralis, but no medullated fibers of these systems reach the body of the cerebellum in this species. Unmedullated correlation fibers, and possibly unmedullated root fibers, pass between the area acustico-lateralis and the cerebellum. The body of the cerebellum receives strong medullated tracts from the spinal cord and oblongata and from the tectum mesencephali, also an unmedullated tract from the hypothalamus (tractus mammillo-cerebellaris). There are two spino-cerebellar tracts, a dorsal and a ventral. The dorsal tract ends chiefly in the body of the cerebellum of the same side; the ventral tract ends partly uncrossed, but most of its fibers enter the cerebellar commissure to reach the body of the cerebellum of the opposite side. These tracts probably also carry ascending fibers from the sensory nuclei of the oblongata to the cerebellum of the same and the opposite side.

The efferent tracts from the cerebellum, so far as demonstrated in *Necturus*, decussate in the ventral commissure and reach the tegmentum of the opposite side of the medulla oblongata and

midbrain, these cerebello-tegmental fibers being strictly comparable with the internal arcuate fibers arising from the entire length of the somatic sensory area of the medulla oblongata. The more anterior members of this system are directed forward and decussate under the midbrain, thus constituting the brachium conjunctivum.

The dorsal decussation of the velum medullare anterius has the following components (cf. fig. 27): (1) fine medullated fibers (*com.cb.*) connecting the corpora cerebelli of the two sides; (2) unmedullate fibers running in the superficial molecular layer and similarly connecting the two corpora cerebelli (*com.cb.l.*); (3) tractus spino-cerebellaris ventralis (*tr.sp.cb.v.*); (4) tractus spino-cerebellaris dorsalis (*tr.sp.cb.d.*), these fibers probably entering the commissure only in very small numbers; (5) fibers of the mesencephalic V root (*mes.V*); (6) fibers of the IV nerve root (*n.IV*). The fifth and sixth of these components have no functional connection with the cerebellum.

I have found no evidence that any root fibers of the V nerve terminate in the cerebellum. The mesencephalic V root traverses the cerebellum in many small bundles, some of which decussate with the cerebellar commissure; but these fibers all appear to pass through into the tectum mesencephali, there to effect connections with the neurones of the nucleus magnocellularis tecti.

The anatomical connections of the cerebellum of lower urodeles conform in general to the scheme of cerebellar organization (statotonus hypothesis) recently set forth by Edinger ('12), though in much simplified form, and there are doubtless many additional details still to be worked out. The relations of the area acustico-lateralis and eminentia ventralis cerebelli of urodeles to the definitive cerebellum of higher vertebrates present problems of considerable interest, whose further investigation is now in process.

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REFERENCE LETTERS

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|---|--|
| <i>a.</i> , dorsal longitudinal correlation tract of area acustico-lateralis | <i>f.sol.</i> , fasciculus solitarius |
| <i>a.ac.</i> , area acustico-lateralis (lobus posterior) | <i>hem.c.</i> , hemisphaeria cerebri |
| <i>b.</i> , ventral longitudinal correlation tract of area acustico-lateralis | <i>l.l.l.</i> , area acustico-lateralis (lobus anterior) |
| <i>br.conj.</i> , brachium conjunctivum | <i>lm.</i> , lemniscus (tr. bulbo-tectalis, somatic sensory) |
| <i>c.cb.</i> , corpus cerebelli | <i>mes.V</i> , radix mesencephalica trigemini |
| <i>com.cb.</i> , commissura cerebelli (medullated component) | <i>n.</i> , neurone of lateral wall of anterior diverticulum of lateral recess |
| <i>com.cb.l.</i> , commissura cerebelli (lateral unmedullated component) | <i>n.IV</i> , nervus trochlearis |
| <i>com.po.</i> , commissura postoptica | <i>n.mot.V</i> , nucleus motorius trigemini |
| <i>com.post.</i> , commissura posterior | <i>nuc.mes.V</i> , nucleus mesencephalicus trigemini |
| <i>com.tect.</i> , commissura tecti | <i>n.V</i> , nervus trigemini |
| <i>d.a.</i> , diverticulum anterior of recessus lateralis | <i>n.VII+VIII</i> , nervi facialis et acusticus |
| <i>em.cb.v.</i> , eminenti ventralis cerebelli | <i>pl.c.</i> , plexus chorioideus |
| <i>em.V</i> , eminentia trigemini | <i>r.l.</i> , recessus lateralis rhombencephali |
| <i>f.ar.</i> , fibrae arcuatae | <i>r.l.l.VII</i> , radix lateralis facialis |
| <i>f.l.m.</i> , fasciculus longitudinalis medialis | <i>r.l.l.X</i> , radix lateralis vagi |
| | <i>r.mot.V</i> , radix motorius trigemini |
| | <i>r.p.m.</i> , recessus posterior mesencephali |

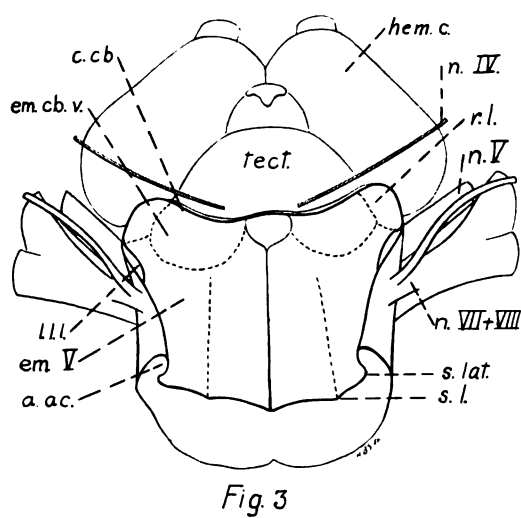
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|---|---|
| <i>r.VII</i> , radix nervi facialis | <i>tr.m.cb.</i> , tractus mamillo-cerebellaris |
| <i>r.X</i> , radix nervi vagi | <i>tr.sp.cb.d.</i> , tractus spino-cerebellaris dorsalis |
| <i>s.a.</i> , stratum album | <i>tr.sp.cb.v.</i> , tractus spino-cerebellaris ventralis |
| <i>s.g.</i> , stratum griseum | <i>tr.sp.t.</i> , tractus spino-tectalis |
| <i>s.l.</i> , sulcus limitans | <i>tr.t.b.</i> , tractus tecto-bulbaris et spinalis |
| <i>s.lat.</i> , sulcus lateralis rhombencephali | <i>tr.th.b.</i> , tractus thalamo-bulbaris |
| <i>tect.</i> , tectum mesencephali | <i>t.v.q.</i> , taenia ventriculi quarti |
| <i>tr.b.t.</i> , tractus bulbo-tectalis (visceral sensory?) | |
| <i>r.m.a.</i> , frenulum veli medullaris anterioris | |



Fig. 1 A view of the right half of the medulla oblongata and cerebellum of *Necturus* seen directly from above and drawn under the stereo-binocular microscope. $\times 16$. See the descriptions of figures 2 and 3.

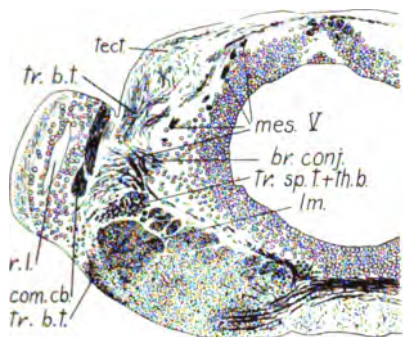
Figs. 2 and 3 Figure 2 is a view of the brain of *Necturus* seen from behind and slightly from above, to illustrate the form of the recessus lateralis rhombencephali and its walls. Fig. 3 is a key drawing to figure 2. $\times 8$. (The peculiar shape of the cerebrum in this figure is due to the fore-shortening resulting from viewing the specimen almost directly from behind. Dorsal, ventral and lateral views of the entire brain of *Necturus* are given by Kingsbury '95, and more detailed dorsal and ventral views by McKibben '13.)

The plexus chorioideus of the fourth ventricle has been removed. At the caudal end of the figure the cross section shows two ependymal sulci in the floor of the ventricle on each side of the medial ventricular sulcus. The inner one of these is the sulcus limitans (*s.l.*), separating the motor area from the sensory area; the lateral one (*s.lat.*) forms the medial boundary of the acoustico-lateral area. The area acustico-lateralis extends forward nearly to the tip of the auricular lobe. At a point between the V and VII roots it is separated by a transverse ependymal sulcus into anterior and posterior lobes (cf. fig. 1). The lateral wall of the recessus lateralis is formed chiefly by the lobus anterior of the area acustico-lateralis and its antero-medial wall by the body of the cerebellum. The walls of the anterior diverticulum are composed of massive but thin cerebellar tissue of very undifferentiated type. The positions of the frenulum of the velum medullare anterius and decussatio veli are indicated by the attachments of the IV nerves.

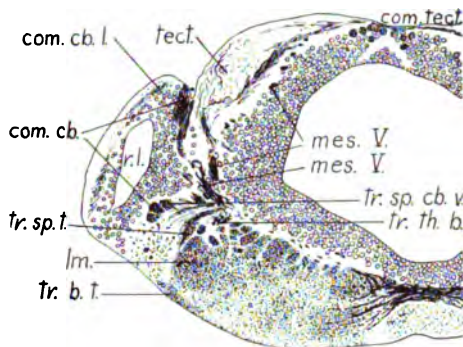


Figs. 4 to 11 Eight transverse sections through the cerebellar region of *Necturus*, arranged in series from a level slightly caudad of the rostral end of the anterior diverticulum of the recessus lateralis rhombencephali (fig. 4) to the superficial origin of the V nerve (fig. 11), to illustrate the arrangement of the chief fiber tracts of this region. $\times 25$.

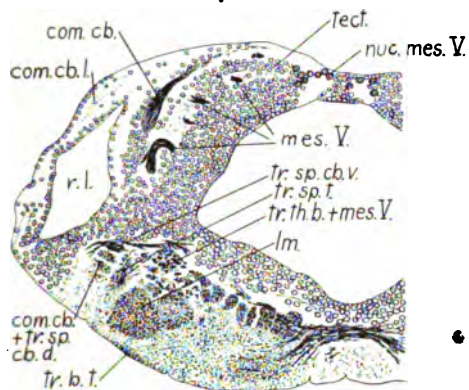
Figures 4, 5, 6, 9, 10 and 11 are drawn from a series of sections prepared by vom Rath's method, and figures 7 and 8 from a series fixed in Zenker's fluid with the acetic acid replaced by 10 per cent formalin and stained by Mallory's method. The specimen from which figures 7 and 8 were drawn is somewhat flattened dorso-ventrally and in both of these specimens the ventricular sulci are less clearly preserved than in some other specimens; cf. figures 12 to 16.



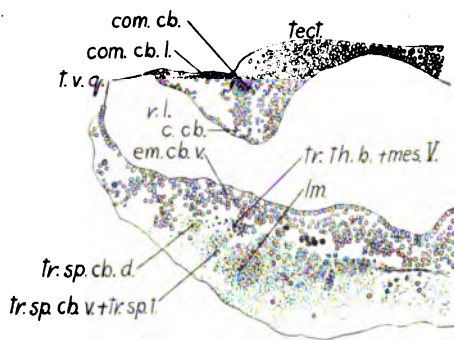
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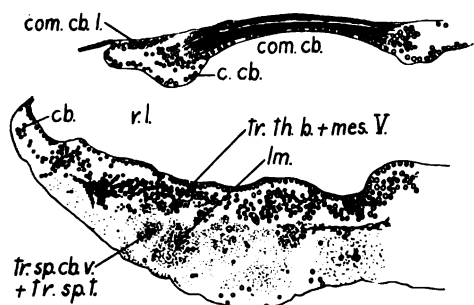
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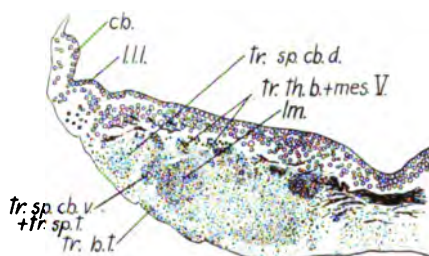
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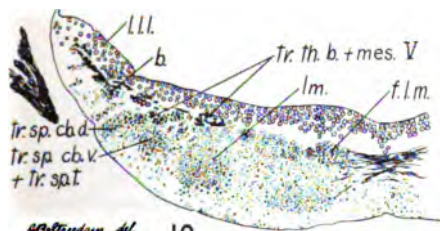
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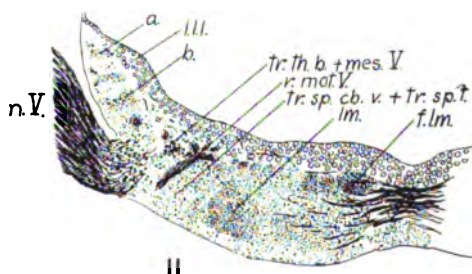
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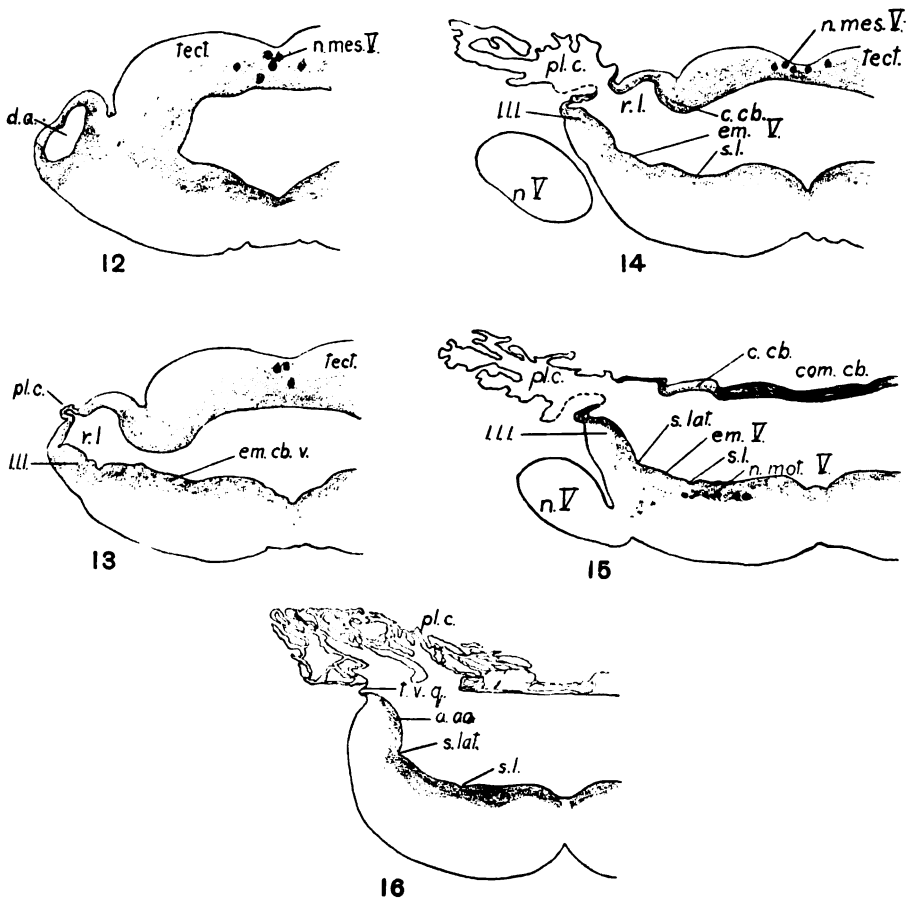
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Figs. 12 to 16 A series of five transverse sections through the brain of *Necturus* fixed in Formalin-Zenker's fluid and stained with carmine, to illustrate the form relations in the cerebellar region. $\times 20$.

This brain was considerably flattened dorso-ventrally during preservation, otherwise the relations are shown correctly.

Fig. 12 Section through the anterior diverticulum of the recessus lateralis rhombencephali.

Fig. 13 Section taken farther caudad, at the level where the massive dorsal wall of the recessus lateralis begins to be replaced by the plexus chorioideus.

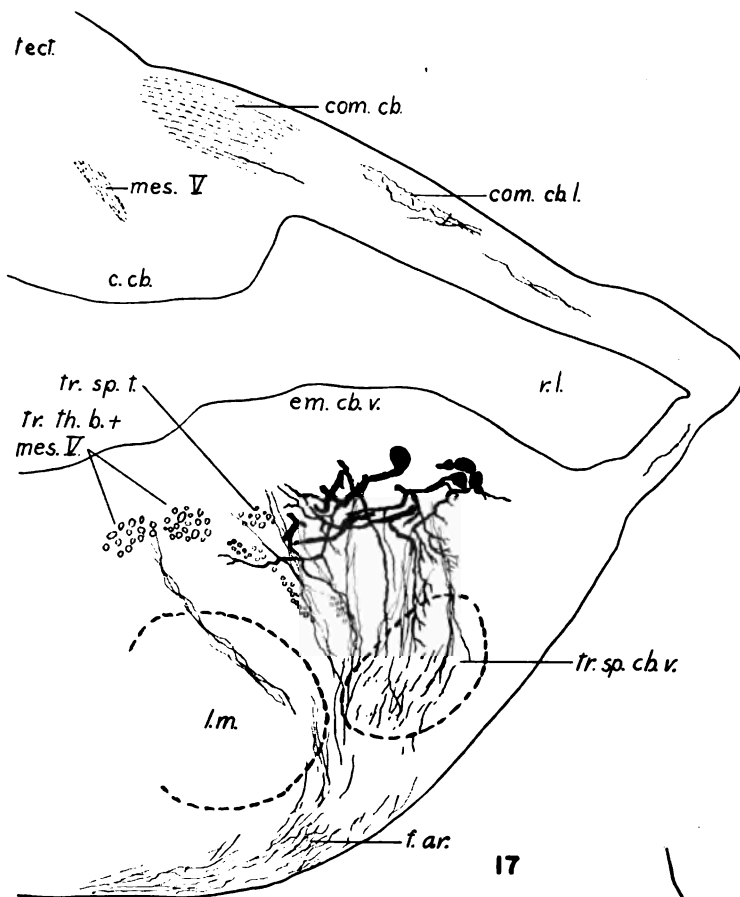
Fig. 14 Section through the caudal end of the recessus lateralis including the rostral ends of the area acustico-lateralis and eminentia trigemini.

Fig. 15 Section at the level of the V roots and commissura cerebelli.

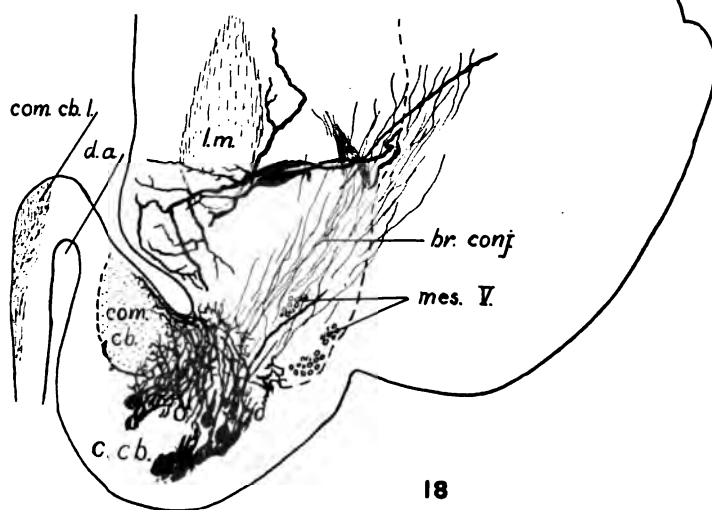
Fig. 16 Section immediately rostrally of the VII roots, showing the relations of the area acustica.

Fig. 17 Transverse section through the right side of the cerebellum and medulla oblongata of *Necturus* at a level slightly rostrad of figure 13, illustrating neurones of the eminentia ventralis cerebelli. Golgi method. $\times 75$.

Fig. 18 Horizontal section through the body of the cerebellum of the left side of the brain of *Necturus*, illustrating neurones of the cerebellum and tegmentum. Golgi method. $\times 75$.



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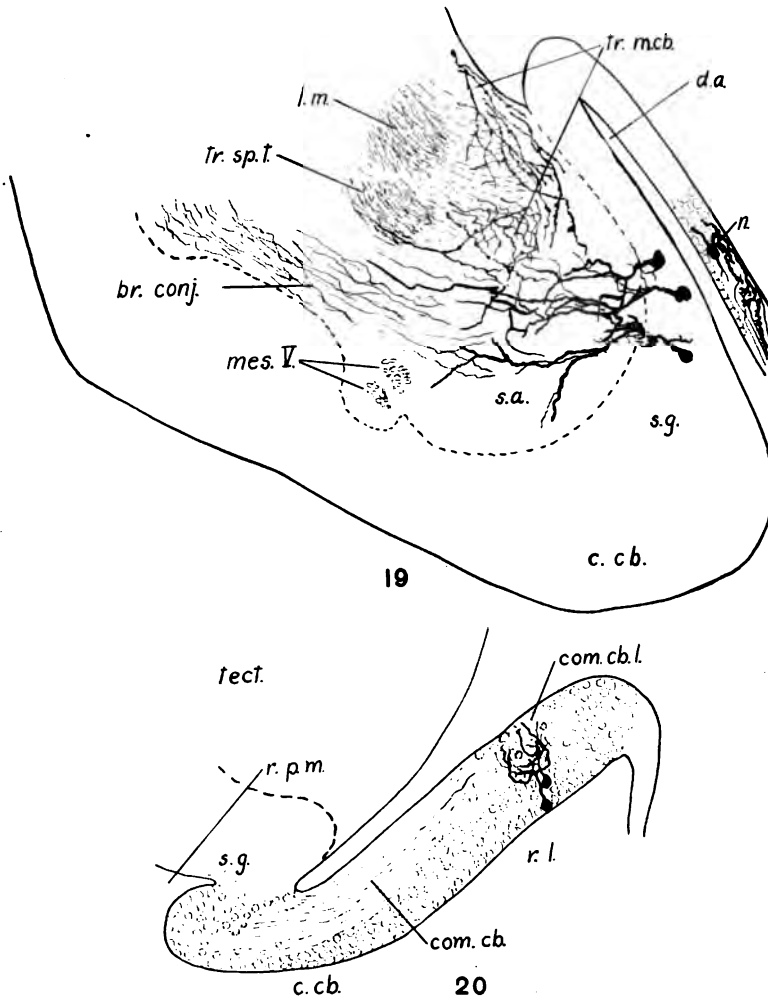
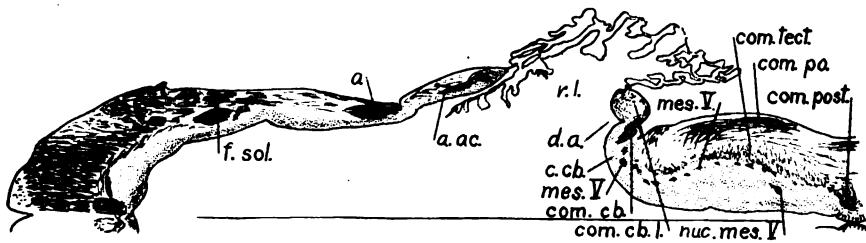


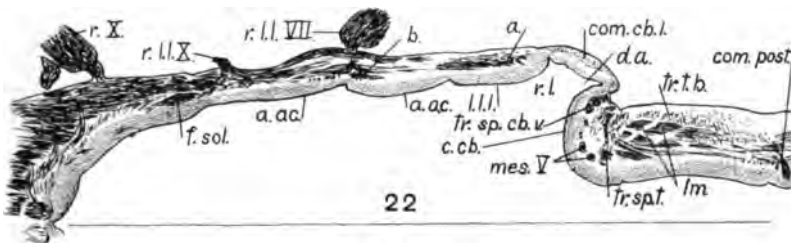
Fig. 19 Horizontal section through the body of the cerebellum of the right side of the brain of *Necturus*, from a different specimen from that shown in figure 18, but in about the same plane, illustrating neurones of the body of the cerebellum and the wall of the anterior diverticulum of the lateral recess. Golgi method. $\times 75$.

Fig. 20 Horizontal section through the most dorsal part of the body of the cerebellum of the right side, of the brain of *Necturus*, illustrating two of its neurones. Golgi method. $\times 75$.

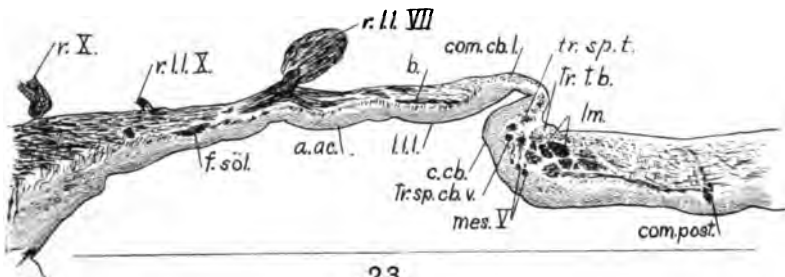
Figs. 21 to 24 Four sketches from a series of horizontal sections through the brain of *Necturus*, stained by Weigert's method. Only the left side is drawn, the horizontal line marking the medial plane. $\times 13$.



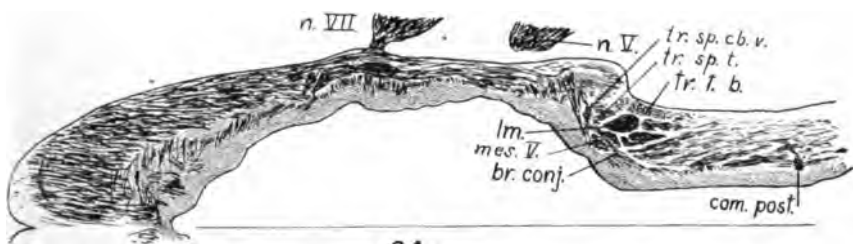
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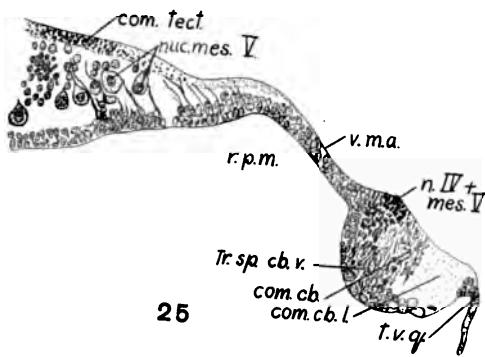
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Fig. 21 Through the dorsal part of the recessus lateralis, illustrating the plexiform lateral wall.

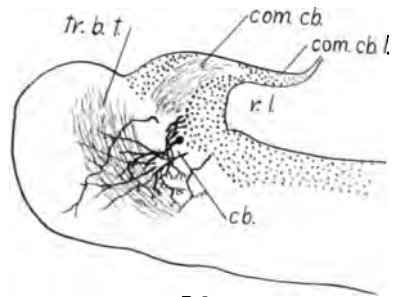
Fig. 22 Section 0.4 mm. farther ventrad.

Fig. 23 Section 0.18 mm. farther ventrad, through the ventral part of the recessus lateralis.

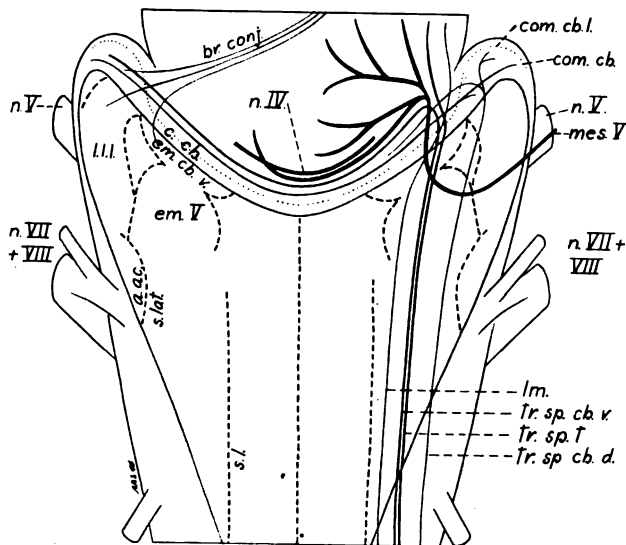
Fig. 24 Section 0.165 mm. farther ventrad, through the floor of the recessus lateralis.



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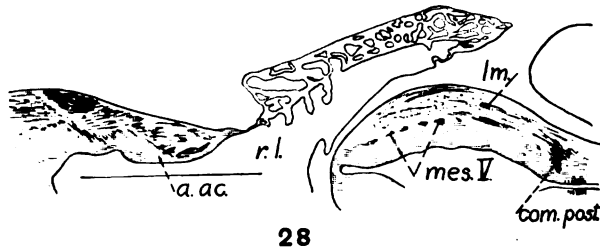
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Fig. 25 Part of a sagittal section through the brain of *Necturus* stained by the Weigert method. The section is strictly medial and includes the caudal end of the tectum mesencephali, the recessus posterior mesencephali with the overlying frenulum of the velum medullare anterius, and the decussatio veli. $\times 75$.

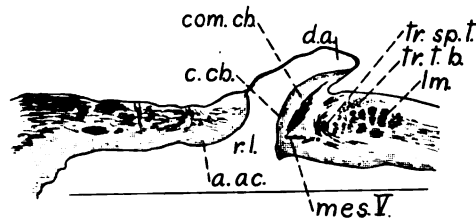
Fig. 26 Parasagittal section through the lateral part of the body of the cerebellum of *Necturus*, illustrating a few neurones whose dendrites are directed forward and downward to engage terminals of the tractus hypothalamo-cerebellaris, cf. figure 19. Golgi method. $\times 30$.

Fig. 27 Diagram of the cerebellar tracts of *Necturus*; cf. figure 1. The tractus cerebello-tegmentalis, tecto-cerebellaris and hypothalamo-cerebellaris are omitted. The tracts here indicated are all medullated wholly or in part, except the commissura cerebelli lateralis (*com.cb.l.*). The connections of the area acustico-lateralis are not included. Unmedullated connections between these structures and the cerebellum may exist, but these have not been determined.

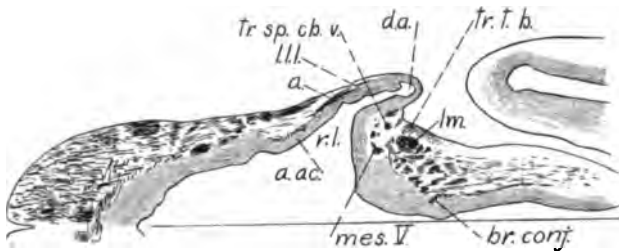
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Figs. 28 to 30 Three horizontal sections through the left side of the brain of *Amphiuma means*. The medial plane is indicated in each case by the horizontal line. $\times 13$.

Fig. 28 Section through the area acustico-lateralis and the chorioid plexus forming the roof of the recessus lateralis rhombencephali.

Fig. 29 Section through the middle of the recessus lateralis. At this level the walls of the recess and of its anterior diverticulum are membranous, though not plexiform, save for the body of the cerebellum in front and the area acustico-lateralis behind. The plexus chorioides which forms the roof of the recess is attached to the parts here illustrated a very short distance dorsally of this level.

Fig. 30 Section through the area acustico-lateralis.

THE STRUCTURE OF THE ROOTS, TRUNK AND BRANCHES OF THE VAGUS NERVE

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TWENTY FIGURES

The work upon which this paper is based was begun by one of us (Chase) on his own initiative. After the preparations were all made, the observations collected in elaborate notes, and the drawings completed, the writing up of the paper fell to the lot of the second author, who has, however, again gone over all the material and writes from first-hand knowledge of the histological details.

It was formerly supposed that the cerebro-spinal nerves (exclusive of the first cerebral) were composed almost entirely of medullated fibers. But it has been demonstrated recently (Ranson '11, '12) that the spinal nerves contain even more non-medullated than medullated fibers. Among the cerebral nerves two have been studied in some detail in recent years. Weigner ('05) found considerable numbers of non-medullated fibers in the *nervus intermedius*. Molhant ('10) found groups of non-medullated fibers in the *vagus*, which he thought were derived from the sympathetic system. The Cajal silver technique was employed by him to stain the axons, and his preparations were evidently unsatisfactory. As a result, his account of the non-medullated fibers in the *vagus* leaves much to be desired. He has, however, given a good account of the medullated fibers in that nerve.

It is not our purpose to present a review of the literature on the *vagus* nerve, since this has been summarized recently by Molhant. Such observations on the structure of the *vagus* as have a direct bearing on those presented in this paper will be summarized in another paragraph.

TECHNIQUE

In dissecting out the vagus nerve in the dog, the bone was removed from around the jugular foramen; and the jugular ganglion, and the vagus and accessory roots were exposed. The common trunk of the vagus and accessory nerves was removed along with their roots and a piece of the medulla oblongata, to which these roots were left attached. The so-called external branch of the accessory was cut away. The superior cervical ganglion of the sympathetic was separated from the carotid plexus and left attached to the vagus by the cervical sympathetic trunk, which in the dog runs in a common connective tissue sheath with the vagus. The inferior cervical ganglion of the sympathetic was left attached to the lower end of this common trunk. The pharyngeal, superior laryngeal and recurrent nerves were dissected out and left attached to the vagus. The vagus trunk in the thorax was removed along with its pulmonary rami and the esophageal plexus. In some dogs the entire vagus with its branches and its connections with the sympathetic was dissected out and preserved. From other dogs only desired portions of the nerve were removed. Most of the preparations were taken from the vagus of the right side.

Although most of the work was done on the dog, the vagus was also studied in man and in the rabbit, cat and rat. The intimate association of the vagus and sympathetic in the dog, which at first sight might appear to complicate the study, is in fact an aid. The sympathetic is always at hand for comparison with the vagus. The two trunks, though contained within one connective tissue sheath in the neck, retain their individuality as completely as in those animals in which they are most widely separated.

Most of the material was cut into serial sections. In this way all branches of the vagus and connections with the sympathetic could be followed accurately. This is especially important in the study of the roots of the vagus, and in the study of the relations of the vagus and sympathetic. Some of the material was fixed in ammoniated alcohol and stained by the pyridine-silver

technique (Ranson '11, '12); some was fixed in Müller's fluid and stained by the Weigert method; and some was fixed and stained in 1 per cent osmic acid. All of the material was imbedded in paraffin and cut into sections varying from 5 to 15 microns in thickness.

THE GROSS ANATOMY OF THE VAGUS IN THE DOG

The vagus nerve in the dog arises by means of a large number of small rootlets from the dorso-lateral sulcus of the medulla oblongata, in series with the roots of the glossopharyngeal and accessory nerves. In figure 1, *a*, the rootlets of the vagus proper are seen running into the jugular ganglion; and, joining the vagus at the side of the ganglion, is seen the spinal root of the accessory nerve *c*. Scattered fine rootlets, *b*, which have come from the medulla, are seen joining the spinal root. These two nerves (vagus and accessory) are fused into a single trunk at the level of the jugular ganglion. Below the jugular ganglion the pharyngeal branch of the vagus and the external branch of the accessory are given off from the common trunk. The superior laryngeal nerve leaves the vagus at the level of the nodose ganglion. Somewhat below the level of this ganglion the sympathetic trunk joins the vagus and runs downward in the same connective tissue sheath with it. Communicating branches from the sympathetic join the pharyngeal and superior laryngeal branches of the vagus. Just above the subclavian artery there is developed in close connection with the vagus the inferior cervical ganglion of the sympathetic. From this the ansa subclavia runs to the ganglion stellatum. From the right vagus, immediately below the inferior cervical sympathetic ganglion, there is given off the recurrent nerve. This receives a branch from the inferior cervical sympathetic ganglion and from it runs a large branch to the posterior cardiac plexus. In the thorax the vagus gives off branches to the root of the lung and is then continued into the esophageal plexus. Here the right vagus, joined by a branch from the left, runs on the right side and posterior aspect of the esophagus to reach the posterior aspect of the stomach. It gives off many fine branches to the esophagus.

NOTES FROM THE LITERATURE ON THE HISTOLOGY OF THE
VAGUS AND ACCESSORY NERVES

Gaskell ('86), working with osmic acid preparations from the dog, found that the accessory nerve was made up of two portions: a larger zone composed chiefly of large medullated fibers with a few medium and small ones, and a smaller zone containing mainly small medullated fibers with a few larger ones and much connective tissue. The fibers in the larger zone were derived from the cervical rootlets. Those in the smaller zone came from the bulbar and highest cervical radicals and went by way of the internal branch of the accessory to join the vagus. Gaskell also found bundles of small medullated fibers in the roots of the vagus and glossopharyngeal nerves. He considers the small medullated fibers in the roots of these three nerves as equivalent to the small fibers in the anterior roots of the spinal nerves, that is, as preganglionic autonomic fibers. From the level of the nodose ganglion downward he found areas in the vagus in which no myelin sheaths were present, and he correctly interpreted these areas as bundles of non-medullated fibers. He assumed that the small preganglionic medullated fibers in the roots lost their myelin sheaths in the nodose ganglion, and thought that they ended there in connection with cells of a sympathetic nature. Gaskell found that the medullated fibers left the vagus by way of the pharyngeal, laryngeal, pulmonary, and cardiac branches; so that very few were left in the vagus at the level of the esophageal plexus.

Molhant ('10) and Van Gehuchten and Molhant ('11), working with the vagus nerve in man and in the rabbit, found three kinds of medullated nerve fibers: large, medium and small. The large fibers with thick myelin sheaths occur chiefly in the pharyngeal branch of the vagus and in the laryngeal branches of the recurrent nerve. Medium-sized fibers mixed with a few large ones are found in the superior laryngeal. Fine medullated fibers and some of medium size are found in the tracheal and esophageal branches of the recurrent nerve and in the thoracic vagus. Molhant, using the Cajal silver stain on the trunk of

the vagus in the rabbit, was able to demonstrate the presence of bundles of non-medullated nerve fibers, which correspond to clear areas in preparations stained by osmic. He believes that these fibers have their origin in the sympathetic system.

Ranvier ('89) found non-medullated fibers in the vagus; but he figured them as branching and forming plexuses. He probably teased out groups of non-medullated fibers.

STRUCTURE OF THE ROOTS OF THE VAGUS AND ACCESSORY NERVES

Osmic acid preparations. As was stated above, the rootlets of the glossopharyngeal, vagus and bulbar portion of the accessory nerve arise in a continuous series from the dorso-lateral sulcus of the medulla. The spinal portion of the accessory arises from the cervical segments of the spinal cord and is joined by the bulbar rootlets. A study of serial sections of the roots of the vagus and accessory nerves, which were removed and fixed with a small piece of the medulla attached, shows the rootlets grouped as in figures 1, 2 and 3. Two complete series of sections were studied. One of these was stained with osmic acid and the other by the pyridine-silver technique. In this way the structure of each set of rootlets was determined.

We will examine first the series stained with osmic acid. At the lower part of figure 2 is seen the large spinal root of the accessory nerve. This has been joined by some of the bulbar rootlets (*b*). The spinal root is composed almost entirely of large medullated fibers with a very few small ones. The fibers are evenly spaced, leaving no clear areas like those seen in some of the rootlets of the vagus. The bulbar portion of the accessory is composed of large and small fibers, the latter predominating. These rootlets are also free from the clear areas seen in some of the radicals of the vagus. Figure 4 is a high-power drawing from one of these bulbar rootlets of the accessory.

The roots of the vagus nerve (fig. 2, *a*) come off from the medulla as a compact group. There may be many fine radicals or one large root and a number of small ones. There are two very different kinds of vagus rootlets. One group, Type I, represented

by radicals 7, 13, 22, 23, 24, 25, 26, 28 and 29, resemble in every respect the rootlets of the bulbar portion of the accessory. That is to say, they are composed of many fine and fewer coarse medullated fibers. They do not have the clear areas seen in the other rootlets of the vagus. The other group, Type II, is represented by rootlets 8, 11, 14, 15, 16, 17, 18, 19, 20 and 21. These contain a considerable number of large and medium-sized medullated fibers and a few small ones. But the characteristic feature of these rootlets is the wide separation of the medullated fibers by unstained tissue. A high-power drawing from one of these rootlets is seen in figure 5, which represents an average field. In some fields the fibers are more widely separated than in the drawing; in other fields they are more closely packed. Even the finest of these medullated fibers stain very sharply and their sheaths are as black as those of the larger fibers. The intervening tissue is light yellow and when highly magnified shows a reticular structure. The stain of even the finest medullated fibers is so perfect that there can be no suspicion of the presence of unstained myelin sheaths in these light yellow areas. Other rootlets, numbers 9, 10 and 12, are mixed in character. They present areas in which the medullated fibers are widely separated, combined with areas in which the medullated fibers, mostly small, are closely packed.

It may be that radicals 7, 8, 9 and 10 correspond to Molhant and Van Gehuchten's group b² or lower vagus rootlets, and the

Fig. 1 Diagram of the vagus nerve in the dog.

<i>a</i> , vagus rootlets	<i>t.s.</i> , truncus sympathicus
<i>b</i> , bulbar rootlets of the accessory nerve	<i>n.v.t.s.</i> , nervus vagus and truncus sympathicus
<i>c</i> , spinal root of the accessory nerve	
<i>g.j.</i> , ganglion jugulare	<i>g.c.i.</i> , ganglion cervicale inferior
<i>r.p.</i> , ramus pharyngeus	<i>art.s.</i> , arteria subclavia
<i>r.e.</i> , ramus externus n. accessorii	<i>n.r.</i> , nervus recurrens
<i>g.n.</i> , ganglion nodosum	<i>a.s.</i> , ansa subclavia
<i>n.l.s.</i> , nervus laryngeus superior	<i>g.s.</i> , ganglion stellatum
<i>n.v.</i> , nervus vagus	<i>r.b.</i> , rami bronchiales
<i>n.c.i.</i> , nervus caroticus internus	<i>p.o.</i> , plexus esophageus
<i>g.c.s.</i> , ganglion cervicale superius	

The drawings were made by Katharine Hill.

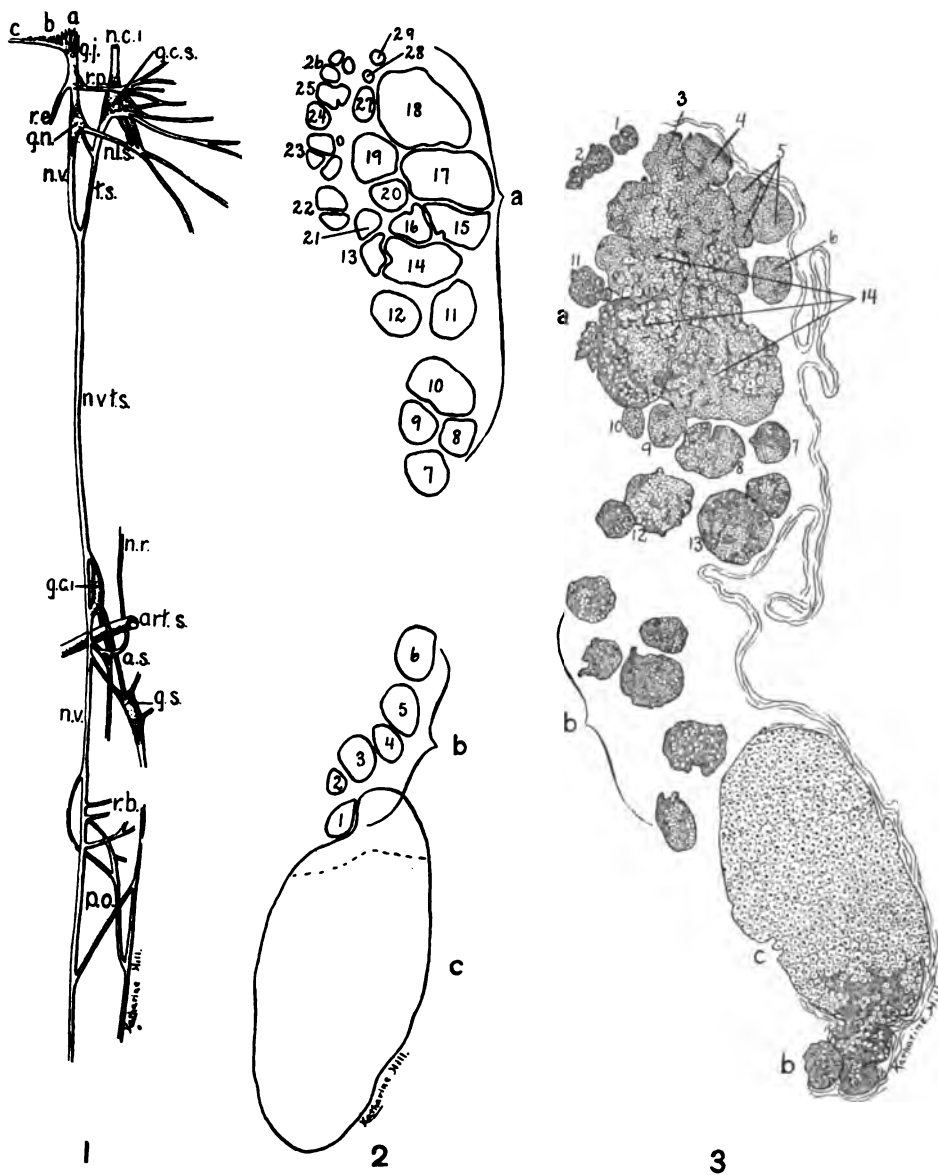


Fig. 2 Outline drawing of the roots of the vagus and accessory nerves from an osmic acid preparation: *a*, vagus rootlets; *b*, bulbar rootlets of the accessory; *c*, spinal root of the accessory. Dog. $\times 27$.

Fig. 3 Roots of the vagus and accessory nerves from a pyridine-silver preparation: *a*, vagus rootlets; *b*, bulbar rootlets of the accessory; *c*, spinal root of the accessory. Dog. $\times 37$.

larger group of radicals, 11 to 29, corresponds to their group b^1 , or higher vagus rootlets. If this be so, their groups b^1 and b^2 , would both contain some of each of our two types of rootlets.

The rootlets of Type II are more closely grouped together than those of Type I and may come off from the medulla as a single large root. Those of Type I are more widely separated from each other and from those of Type II.

To sum up the findings in osmic acid preparations of the roots of the vagus and accessory nerves, the following statements concerning their content of medullated fibers may be made. The spinal root of the accessory nerve is composed almost wholly of large fibers of uniform size. The bulbar portion of the same nerve is composed largely of those of the smallest size, with some large ones, and fewer of medium size. The vagus rootlets are of two kinds: those of Type I have the same structure as the bulbar portion of the accessory; those of Type II contain medullated fibers of all sizes, with a predominance of the medium-sized, separated by a large amount of unstained tissue.

Pyridine-silver preparations. The pyridine-silver method stains only the axons of the nerve fibers. These vary from black in the non-medullated to light yellow in the large medullated fibers. The connective tissue is also stained a light yellow. Osmic acid

Fig. 4 From a section of a bulbar rootlet of the accessory nerve stained with osmic acid. Dog. $\times 515$.

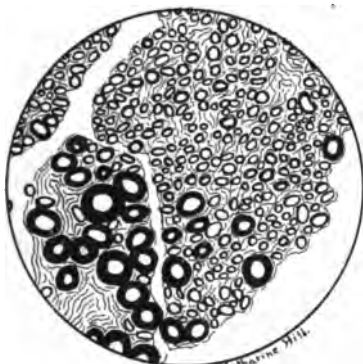
Fig. 5 From a section of a Type II rootlet of the vagus nerve stained with osmic acid. Dog. $\times 515$.

Fig. 6 From a section of the vagus nerve below the nodose ganglion stained with osmic acid. Dog. $\times 618$.

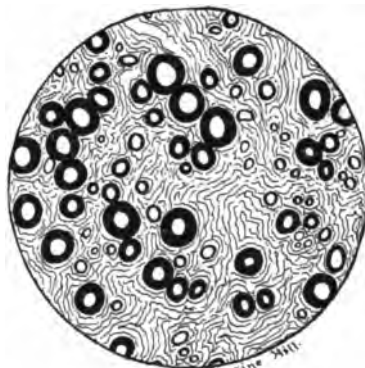
Fig. 7 From a section of the superior laryngeal nerve stained with osmic acid. Dog. $\times 618$.

Fig. 8 From a section of the recurrent nerve stained by the pyridine-silver technique: *a*, area of large medullated fibers for the larynx; *b*, area of small medullated fibers for esophagus, trachea, and heart; *c*, area of sympathetic fibers. Dog. $\times 176$.

Fig. 9 From a section of the common vagus-accessory trunk at the level of the jugular ganglion; the accessory nerve at the right, the jugular ganglion at the left, the vagus in the middle. The section of the accessory nerve shows two well defined areas: the lower, lighter area corresponds to the spinal root; the upper, darker area corresponds to the bulbar rootlets. Pyridine-silver. Dog. $\times 73$.



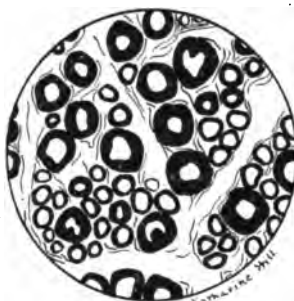
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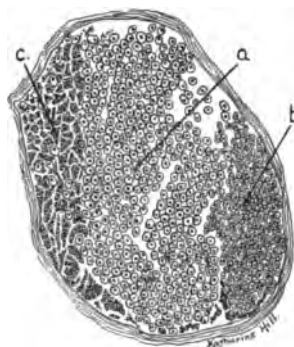
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and pyridine-silver preparations give mutually supplementary pictures. Large and medium-sized medullated axons are easily recognized in the silver preparations by the clear unstained rings of myelin which surround them. In order to make sure of the differentiation between the finest medullated and the non-medullated fibers, it is necessary to supplement the silver preparations by those stained with osmic acid.

In figure 3 is shown a drawing of the roots of the vagus and accessory nerves taken at about the same level as figure 2. The relative position of the individual rootlets is much the same in the two figures. Pyridine-silver preparations confirm the findings in those stained with osmic acid, in so far as the medullated fibers are concerned.

The light yellow axons of the large medullated fibers, surrounded by thick unstained rings of myelin, give the spinal root of the accessory nerve (c) a light color. The bulbar rootlets of the accessory (b) are stained more darkly, because the small medullated axons of which the roots are chiefly composed stain a dark brown. In these preparations one can readily recognize the thin clear rings of myelin surrounding the finest of the medullated fibers. There are few, if any, non-medullated fibers in the roots of the accessory nerve.

The two types of vagus rootlets are clearly differentiated in pyridine-silver preparations. Those of Type I (Nos. 1 to 7 and 11) have the same structure as the bulbar rootlets of the accessory, and contain many small and fewer large medullated fibers and very few, if any, non-medullated axons. It will be seen that these rootlets are arranged somewhat differently than in figure 2.

The large root, 14, came off the medulla as a single trunk. The fiber bundles on its left hand border are of mixed character. But the rest of the large root 14 and the rootlets 8, 9, 10 and 12 are of Type II. In these the medullated fibers are of all sizes rather widely separated from each other. In the areas left vacant by the medullated fibers are seen enormous numbers of axons stained black and imbedded in the light yellow endo-

neurium (fig. 12). These bundles of non-medullated axons correspond to the unstained areas in the osmic acid preparations (fig. 5). While these axons are evenly distributed in some portions of the large root, they have a tendency to be grouped into bundles of from 5 to 50 or more.

The fiber bundles on the left hand border of the large root (14) are of mixed character with the fine medullated fibers of the Type I rootlets predominating. Number 13 is also of mixed character, being mostly of Type I but containing two small areas of Type II. Figure 13 was drawn from this fascicle and shows one of the two groups of non-medullated fibers situated at the periphery of the radical. These are scattered among large and medium-sized medullated fibers. Elsewhere in the drawing the fine medullated fibers predominate. The two areas in figure 13 have the structure of the Type II and the Type I rootlets respectively.

Pyridine-silver preparations, then, correspond to the osmic acid preparations so far as the number, size and distribution of the medullated fibers in the various rootlets are concerned. In addition, they show the presence in the vagus rootlets of Type II of enormous numbers of non-medullated axons. These occupy the areas which are unstained in osmic acid preparations. They are many times more numerous than the medullated fibers in the rootlets of this type.

So far as we know, these fibers have not been observed before in the roots of the vagus. Molhant saw them in the trunk of this nerve; and Gaskell inferred their presence here, basing his inference on the unstained areas in osmic acid preparations. Their presence in the rootlets is of special importance, since it shows that those found in the trunk are not sympathetic fibers as was maintained by Molhant. They belong properly to the vagus nerve.

We wish to call attention to the striking similarity between the vagus rootlets of Type I and the efferent roots of the spinal nerves, and between the vagus rootlets of Type II and the afferent roots of the spinal nerves. The efferent spinal nerve roots

contain few, if any, non-medullated fibers, and differ from the rootlets of Type I only in containing a much smaller proportion of fine medullated fibers. This difference is easily understood when one remembers that the vagus innervates the thoracic and abdominal viscera, and its efferent rootlets should contain an excessive number of fine autonomic fibers. The afferent roots of the spinal nerves contain, in addition to medullated fibers of all sizes, large numbers of non-medullated axons, that is, essentially the same elements as are found in the vagus rootlets of Type II. The proportion of non-medullated fibers is undoubtedly greater in these rootlets of the vagus than in the dorsal roots of the spinal nerves; but otherwise the similarity is complete. In view of these facts it seems probable that the afferent and efferent fibers of the vagus leave the medulla by separate rootlets. In the mixed rootlets the areas representing each of the two types are sharply marked off from each other and are formed by the fusion of one or more smaller rootlets of each type.

Fig. 10 From a section of the common vagus-accessory trunk below the level of the jugular ganglion; the spinal portion of the accessory to the right, the vagus to the left and the bulbar portion of the accessory in the middle. Pyridine-silver. Dog. $\times 73$.

Fig. 11 From a section of the right common vagus-sympathetic trunk just above the level of the subclavian artery. The crescentic area in the lower part of the drawing is the sympathetic trunk. The bundle of large medullated fibers in the upper part of the drawing represents the beginning of the recurrent nerve. Pyridine-silver. Dog. $\times 73$.

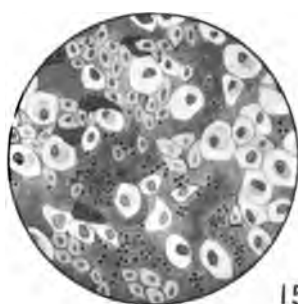
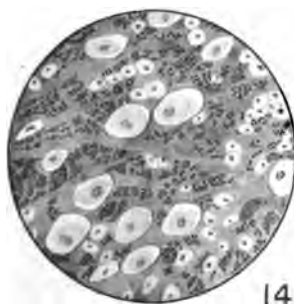
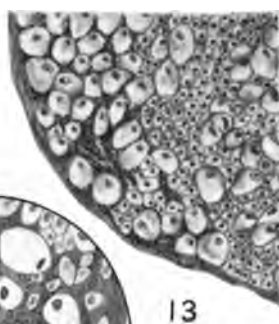
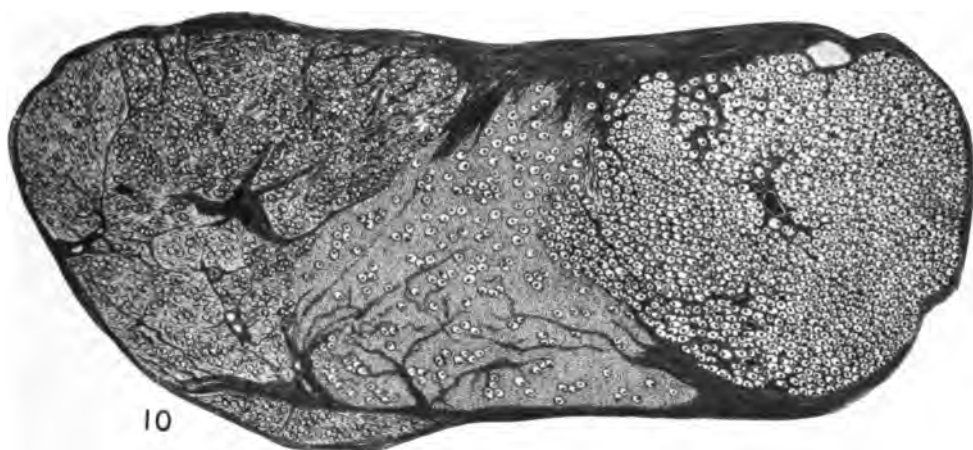
Fig. 12 From a section of a Type II vagus rootlet. Medullated axons light, myelin sheaths colorless, non-medullated fibers black. Pyridine-silver. Dog. $\times 618$.

Fig. 13 From a section of a mixed vagus rootlet. At the left near the periphery is an area having the structure of the Type II rootlets and containing, in addition to medullated fibers of all sizes, large numbers of non-medullated axons. To the right is an area having the structure of the Type I rootlets, containing chiefly small medullated fibers. Pyridine-silver. Dog. $\times 618$.

Fig. 14 From a section of the vagus below the level of the nodose ganglion. The medullated fibers are of all sizes and are widely separated by great numbers of non-medullated axons. Pyridine-silver. Dog. $\times 618$.

Fig. 15 From a section of the superior laryngeal nerve, medullated and non-medullated fibers. Pyridine-silver. Dog. $\times 618$.

Fig. 16 From a section of a fascicle of the esophageal plexus. Six medullated and great numbers of non-medullated fibers. Pyridine-silver. Dog. $\times 739$.



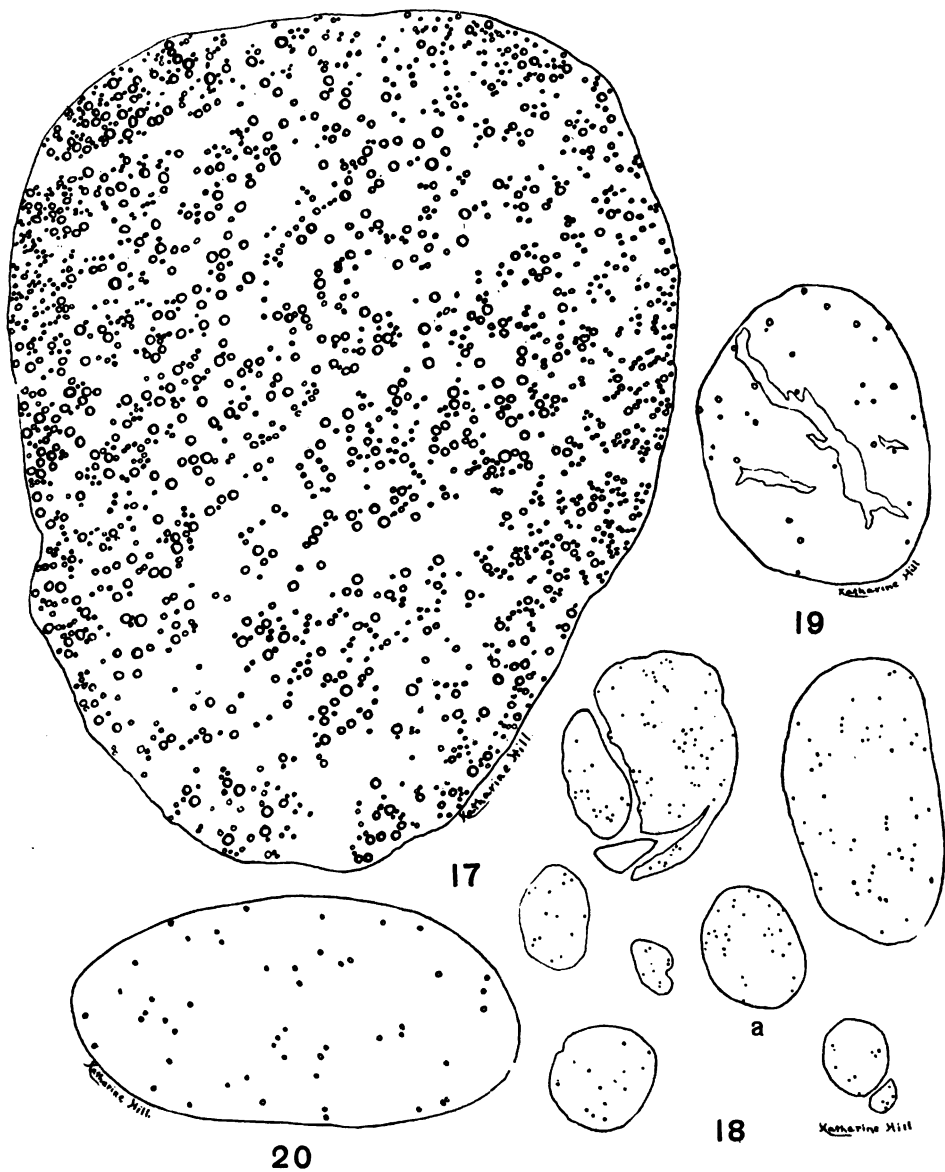


Fig. 17 From a section of the vagus above the level of the pulmonary rami. Osmic acid. Cat. $\times 221$.

Fig. 18 From a section through the esophageal plexus. Osmic acid. Dog. $\times 85$.

Fig. 19 Fascicle, *a*, of figure 18 more highly magnified. $\times 221$.

Fig. 20 From a section of the vagus at the level of the diaphragm. Osmic acid. Cat. $\times 221$.

STRUCTURE OF THE VAGUS AND ACCESSORY NERVES AT THE
LEVEL OF THE JUGULAR GANGLION

Traced distally, the roots described in the preceding section converge toward the jugular foramen. The rootlets of the vagus fuse into a single trunk; and the bulbar rootlets of the accessory one after another join the spinal root of that nerve. All these bulbar rootlets join the same side of the spinal root on the surface of which they form a crescentic field. The fibers in this bulbar area do not mix with those of the spinal portion.

After this fusion has taken place, there are two trunks, the vagus and accessory nerves, running side by side. Shortly after the vagus rootlets have united into a single trunk the jugular ganglion appears. It first is seen as a group of cells in the connective tissue at one side of the nerve (fig. 9). In the succeeding sections groups of ganglion cells appear within the fascicles of the nerve trunk; and for some distance downward columns of ganglion cells are seen in the nerve. Kölliker ('96) says that, whereas in man the vagus goes with all its roots into the jugular ganglion, in the dog a small part of the nerve remains separate from the ganglion. Macroscopically this might seem to be the case, but microscopic sections show cells of the jugular ganglion at some level in practically all parts of the trunk. It is impossible to say that any bundle of nerve fibers is entirely free from nerve cells. The jugular ganglion is, then, represented in the dog by an accumulation of cells on one side of the nerve and by cells scattered more or less diffusely through it.

Bundles of nerve fibers can be seen passing from the laterally-placed accumulation of ganglion cells into the nerve trunk. In these bundles one sees some non-medullated fibers. This indicates that some of the non-medullated fibers seen in the roots arise from cells in the jugular ganglion, just as the non-medullated fibers in the dorsal roots arise from cells of the spinal ganglia.

In tracing the roots distally in the serial sections, one sees that, at a level slightly below the beginning of the jugular ganglion, the vagus and accessory nerves unite to form a single trunk. The accessory nerve is shown in the right third of figure 9. The

darker area in the upper right-hand corner of the drawing is the bulbar portion of the accessory; the lighter area in the lower right-hand corner is the spinal portion of the accessory. The spinal portion of the accessory remains unchanged and forms a sharply defined zone in the common vagus-accessory trunk. At no level in the series do its fibers mingle with those of the other parts of the common trunk. The bulbar rootlets of the accessory have fused and now form a sharply limited area wedged in between the spinal part of the accessory and the vagus. Thus, although the two nerves are fused into a common trunk, it is possible to distinguish clearly three areas, corresponding to the spinal root of the accessory, the bulbar roots of the accessory, and the roots of the vagus. Each area presents the same histological characteristics as the corresponding roots at a higher level. The only difference is that the non-medullated fibers are more evenly distributed in the vagus at this level than they were in the roots, since the fibers from the two types of vagus rootlets are now thoroughly mixed.

THE VAGUS AND ACCESSORY NERVES BETWEEN THE JUGULAR AND NODOSE GANGLIA

Below the jugular ganglion the vagus-accessory trunk may be broken up into a large number of fascicles, but these are again reunited into a compact trunk from which the ramus externus of the accessory is given off. In one series the trunk did not break up into fascicles between the jugular ganglion and the origin of the external ramus. Figure 10 is a drawing from this series just above the level at which the external ramus is given off. The three zones seen in figure 9 are also seen in figure 10; the spinal portion of the accessory to the right, the vagus to the left, and the bulbar portion of the accessory in the middle. In those cases in which the common trunk broke up into fascicles below the jugular ganglion the fascicles again reunited to form a trunk essentially like that in figure 10. The bulbar portion of the accessory was not so sharply separated from the vagus at this level in all the nerves studied, but was in some cases scattered through the vagus.

The structure of the external branch of the accessory nerve as it leaves the common trunk is the same as that of the spinal root. In fact, as has been seen by the study of these serial sections, the spinal root maintains its individuality throughout the common trunk, where it runs as a well-defined bundle, and leaves the common trunk as the ramus externus. The vagus and the bulbar portion of the accessory have the same structure as at a higher level but are less sharply separated from each other.

Below the level of the origin of the external branch of the accessory the vagus trunk, which now contains the bulbar fibers of the accessory, may continue for a short distance as a single trunk. It is usually, however, at once broken up again into fascicles. Or perhaps it would be better to say that many small fascicles are given off which may branch and anastomose with the main trunk. The complexity of this plexus formation differs in different individuals. From one or more of these fascicles of the vagus just above the level of the nodose ganglion the pharyngeal branch is given off.

In the vagus just below the jugular ganglion there are clear areas in osmic acid preparations like those seen in the rootlets of Type II. Pyridine-silver preparations show that these clear areas are filled with non-medullated fibers. Molhant has made similar observations on the structure of the vagus at this level in the rabbit.

THE VAGUS AT THE LEVEL OF THE NODOSE GANGLION

In all cases the fascicles begin to unite into fewer bundles as the nodose ganglion is approached. In some cases all of the fascicles are united into a single trunk before the ganglion appears. In others the ganglion cells appear in one or more of the still separate fascicles which are then united in the ganglion. The nodose ganglion presents the appearance of a fusiform swelling of the nerve trunk; and apparently all the fiber bundles are involved.

In the dog the bulbar fibers of the accessory become intimately mingled with the vagus fibers at or above the level of the nodose ganglion. In the rabbit, according to Molhant, the fascicles

containing the bulbar fibers remain for the most part outside of the nodose ganglion and mingle with the vagus fibers below it. In some rabbits he found the vagus and bulbar accessory fibers intermingled above the level of the nodose ganglion.

From what has been said in this and preceding paragraphs it will be apparent that no such thing as the internal branch of the spinal accessory exists in the dog. According to Van Gehuchten and Molhant, it is also absent in man and in the rabbit. The old description of the accessory dividing into an internal and external branch, the former of which joins the vagus, is based on artificial conditions produced by the dissector's knife. The vagus and accessory nerves fuse into a common trunk at the level of the jugular foramen. The so-called spinal part maintains its individuality throughout this common trunk, beginning as the spinal root and ending as the external branch. Composed as it is almost entirely of large medullated fibers it differs markedly from the rest of the common trunk. The bulbar portion, composed chiefly of fine medullated fibers, maintains its individuality for a varying distance in the common trunk and then mingles with the fibers from the vagus rootlets. Since the bulbar fibers of the accessory have an origin from the medulla similar to that of the vagus fibers, since they form rootlets exactly like the vagus rootlets of Type I, and since they become fused with the vagus trunk and distributed in its branches, it would be more proper to speak of the bulbar portion of the accessory as a part of the vagus which for a short distance runs in company with the accessory nerve.

In serial sections through the upper part of the ganglion a large bundle of good-sized medullated fibers can be seen collecting at one side. At about the middle of the ganglion this bundle leaves the vagus as the superior laryngeal nerve. The structure of this and the pharyngeal branch will be taken up in another place.

In the nodose ganglion the number of non-medullated fibers appears to be much greater than at higher levels. This may be due to an increased intensity of staining bringing out fibers that were unstained at a higher level. It is, no doubt, in part due

to the fact that the pharyngeal and superior laryngeal nerves take out large numbers of medullated fibers from the vagus trunk, allowing the non-medullated fibers to be more closely grouped together. It may in part be due to a real increase in the number of these fibers. Gaskell, who failed to note the presence of non-medullated fibers in the roots of the vagus, but found them in the trunk, concluded that the small medullated fibers in the roots of the vagus and bulbar portions of the accessory ended about cells of the sympathetic type in the ganglion nodosum, and that non-medullated fibers arose from these cells and were continued downward in the trunk of the vagus. Thus he accounted for the non-medullated fibers of the trunk of the vagus as post-ganglionic autonomic fibers and made the nodose ganglion an autonomic ganglion.

Langley ('03) presents evidence to show that post-ganglionic fibers do not begin in the nodose ganglion, and that all pre-ganglionic fibers of the vagus end about the cells of ganglia in the peripheral plexuses associated with the vagus. In Schäfer's textbook of physiology Langley says that the efferent autonomic fibers of the vagus and the accessory nerves are apparently not connected with the cells of the jugular or nodose ganglia. It is probable that the nerve fibers for each organ end in small ganglia situated in or near the organ itself. He calls attention to Gaskell's observations, that non-medullated fibers appear in the vagus immediately below the nodose ganglion, and concludes that since these can not be post-ganglionic fibers it must follow that either the afferent fibers become non-medullated a long way from their endings, or that the pre-ganglionic fibers lose their sheaths as they pass downward in the vagus. In the sympathetic system, he says, it can hardly be doubted that pre-ganglionic fibers become non-medullated a considerable distance from the cells to which they run. We may fairly assume that the same may be the case with the similar fibers of the vagus.

It will be seen that the anatomical data as stated by Gaskell and utilized by Langley in this argument are not strictly correct, since non-medullated fibers are also found in great numbers in the roots of the vagus. But, if the apparent increase in the num-

ber of non-medullated fibers in the vagus at the level of the nodose ganglion proves to be real, Langley's argument would help us account for this increase. It is not unreasonable to suppose that the pre-ganglionic fibers of the vagus may lose their sheaths in the trunk of the vagus, just as the pre-ganglionic fibers of the sympathetic lose their sheaths at a distance from the cells about which they end.

THE VAGUS BELOW THE NODOSE GANGLION AND ITS RELATION TO THE SYMPATHETIC TRUNK

The vagus may remain as a single trunk throughout the nodose ganglion; or toward the lower pole of the ganglion it may begin to break up into fascicles with ganglion cells in one or more of them. Usually the vagus is broken up into several fascicles just below the ganglion. These divide and unite with one another forming a complicated plexus. There is no regularity in these formations. The nerves in different individuals present marked variations.

There is no essential change in the histology of the vagus immediately below the nodose ganglion. The non-medullated fibers are more conspicuous and appear to be present in somewhat greater relative proportions. The medullated fibers are of all sizes and are rather widely separated (fig. 6). The intervening tissue is unstained in osmic acid preparations, and looks like connective tissue. But pyridine-silver preparations show that this intervening tissue is filled with non-medullated axons (fig. 14). They are present in astonishingly large numbers and show a tendency to be grouped into bundles.

Just before the right nerve passes over the subclavian artery there is formed at one side a fascicle of large medullated fibers (fig. 11). These finally leave the vagus as the recurrent nerve.

In figure 11 is seen a cross-section of the common trunk of the vagus and sympathetic in the neck. The sympathetic trunk is flattened out on the surface of the vagus and its area in section is crescentic. While the sympathetic trunk in the dog is usually flattened out on the vagus at this level, it may remain throughout

in the neck as a rounded fascicle, separated from the vagus by a connective tissue septum but contained with the vagus in a common connective tissue sheath. Even when flattened out on the vagus the sympathetic is usually separated from the vagus by a thin connective tissue septum. Holzmann and Dogiel ('10) found that in some dogs the sympathetic trunk was easily dissected from the vagus. These must have been cases in which it remained as a separate rounded fascicle.

Following the sympathetic trunk upward in the neck we find that it parts company with the vagus at a variable distance below the nodose ganglion, and runs as a separate trunk to the superior cervical ganglion. Just below this ganglion it contains both fine medullated and non-medullated fibers. The fine medullated fibers are ascending pre-ganglionic fibers from the thoracic spinal nerves and are very numerous. As the sympathetic trunk is traced into the ganglion the medullated fibers are lost among the ganglion cells.

The presence of large numbers of non-medullated fibers in the vagus of the dog suggests the possibility of an admixture of sympathetic fibers because of the close relation of these two nerves in that animal. This raises the question as to whether the observations here recorded are applicable to the human vagus which is not included in a common connective tissue sheath with the sympathetic trunk. A short stretch of the human vagus just below the nodose ganglion was removed within a few hours after death and stained by the pyridine-silver method. This presents essentially the same picture as the cervical vagus in the dog. The chief difference being that the non-medullated fibers are more evenly distributed throughout the nerve in the dog. In the human vagus they tend to form larger bundles and there are some bundles of medullated fibers quite free from them. But so far as one can tell, the relative proportion of the two kinds of fibers is about the same in the dog and in man.

Several sets of serial sections were gone over carefully to determine to what extent sympathetic fibers enter into the composition of the vagus in the dog. The two trunks, although

bound together in a common sheath in the neck, remain independent and distinct. In each series some few very fine sympathetic filaments could be traced into the vagus; but no communicating twigs of large size were seen passing between these two nerves in the common sheath. On the other hand, free communications between the pharyngeal and laryngeal branches and the sympathetic were found. Some fairly good-sized bundles of sympathetic fibers join these branches and run along them to join the vagus trunk. These sympathetic bundles can be recognized easily in the vagus, where they retain their individuality and are differentiated from the vagus fibers by their lighter stain and the compactness with which the fibers are grouped. These sympathetic bundles can be followed long distances in serial sections as well-defined independent bundles within the vagus. These sympathetic bundles can sometimes be followed into the vagus, along its trunk, and out again without their having in any way lost their identity.

It thus appears that the cervical trunk of the vagus in the dog, although contained for some distance in a common sheath with the sympathetic, is not especially intimately connected with that nerve; and the number of sympathetic fibers that enter the vagus is insignificant in comparison with the total number of its non-medullated fibers. So far as we have been able to determine, these sympathetic fibers are all contained within well-defined bundles which run with the vagus for a short distance to leave it again at another level.

THE VAGUS AT THE LEVEL OF THE INFERIOR CERVICAL GANGLION OF THE SYMPATHETIC

In the lower part of the neck the sympathetic leaves the vagus to run into the inferior cervical ganglion. This ganglion is connected with the vagus trunk by numerous small and a few good-sized bundles of sympathetic fibers. The lower pole of the ganglion is attached to the vagus and to the beginning of the recurrent laryngeal nerve by one or more good-sized fascicles. These represent the lower half of the ansa subclavia and leave the vagus

at a slightly lower level to run to the stellate ganglion (fig. 1). They are incorporated in the vagus trunk but retain their individuality and do not mix with the vagus fibers. The communications between the recurrent and sympathetic nerves at this level are very complicated and we have not been able to work them out satisfactorily. While it is clear that most of the sympathetic fibers that join the vagus in the region of the inferior cervical ganglion remain in sharply defined bundles and soon leave the vagus again, it is not possible to be sure that there is not some intermingling of vagus and sympathetic fibers.

THE PHARYNGEAL BRANCH OF THE VAGUS

Just above the level of the nodose ganglion the vagus is broken up into several fascicles, as was mentioned in an earlier paragraph. These fascicles branch and reunite forming a complicated plexus. The pharyngeal branch arises from one or more of these fascicles. It was not possible to trace the origin of this branch from the bulbar fibers of the accessory, as was done by Molhant in the rabbit. These accessory fibers are so intimately mixed with the vagus fibers in the plexus just described that it is not possible from a study of serial sections in the dog to state whether the fibers of the pharyngeal branch are derived from the bulbar roots of the accessory or not.

Osmic acid preparations show that the pharyngeal branch is composed for the most part of large medullated fibers, but contains also a considerable number of medium and small medullated fibers. Pyridine-silver preparations show that there are few, if any, non-medullated fibers in this branch.

THE SUPERIOR LARYNGEAL NERVE

In both osmic acid and silver preparations a large bundle of medullated fibers can be seen leaving the vagus at about the middle of the ganglion nodosum. This is the superior laryngeal nerve. Osmic acid preparations (fig. 7) show that it contains large, medium and small medullated fibers, with the medium

and small-sized fibers predominating. In pyridine-silver preparations (fig. 15) non-medullated fibers are seen in considerable numbers; but they are much less numerous than in the vagus trunk and are less evenly distributed.

THE RECURRENT NERVE

In the lower part of the cervical trunk of the vagus a number of large medullated fibers become grouped into a bundle near the periphery of the nerve (fig. 11). This bundle separates off a little lower down to form the recurrent nerve. At its origin this nerve is composed of two quite sharply defined areas: one (fig. 8, *a*) represents the bundle of large medullated fibers mentioned above; the other (fig. 8, *b*) contains small and medium-sized medullated fibers. In figure 8 there is also seen a third area, *c*, formed of sympathetic fibers. The recurrent nerve at its origin from the vagus is closely associated with the branches of the inferior cervical sympathetic ganglion.

Pyridine-silver preparations show that there are few, if any, non-medullated fibers in the recurrent nerve aside from this obviously sympathetic bundle that accompanies it. Mqlhant found that the smaller fibers of the recurrent nerve, that is, those in bundle *b*, were distributed in the cardiac, esophageal and tracheal branches, and that the nerve as it enters the larynx is made up entirely of large medullated fibers. Our preparations confirm these observations.

THE VAGUS BELOW THE ORIGIN OF THE RECURRENT NERVE

As has already been stated, the vagus, is very closely connected with the sympathetic trunk at the level of the inferior cervical ganglion and at the origin of the recurrent nerve. Below the level of the recurrent nerve the lower loop of the ansa subclavia runs with the vagus for a short distance. The thoracic vagus, after the ansa subclavia has separated from it, has the same structure as the cervical trunk, except that it contains an even greater proportion of non-medullated fibers. This is in part accounted for by the fact that the recurrent nerve has taken out most of the

remaining large medullated fibers. The pharyngeal, superior laryngeal, and recurrent nerves take out from the vagus large numbers of medullated fibers, while of these only the superior laryngeal contains appreciable numbers of non-medullated fibers, and even this nerve contains relatively few of them. It will be seen, therefore, that practically all of the non-medullated fibers in the cervical vagus are carried down with the thoracic vagus, where they are more compactly grouped, due to the fact that the larger part of the medullated fibers have left the vagus in its cervical branches. This explains in part, at least, why the thoracic vagus contains a much greater proportion of non-medullated fibers than does the upper cervical trunk.

It is doubtful if this accounts for the entire increase in the proportion of non-medullated fibers in the thoracic vagus. It may be that there has been some admixture of sympathetic fibers. It seems probable that an important factor in this increase is a change in the character of the pre-ganglionic fibers. It seems probable that some of these lose their sheaths as they pass down the vagus. Langley's argument in favor of this has been given in a preceding paragraph.

Figure 17 is a drawing of an osmic acid preparation of the thoracic vagus in the cat above the level of the pulmonary rami. It shows that there are a few large medullated fibers, but that the majority of the medullated fibers are either small or of medium size. The most noticeable feature is the wide spacing of these fibers and the large amount of intervening unstained substance. In pyridine-silver preparations these unstained spaces are seen to be filled by myriads of non-medullated axons.

THE VAGUS BELOW THE BRONCHIAL RAMI

As the vagus passes behind the root of the lung it gives off several branches to the pulmonary plexuses. These bronchial rami contain large numbers of medullated fibers and take from the vagus a considerable proportion of those medullated fibers which have continued down the vagus to this level. We have no data as to the presence or absence of non-medullated fibers in

these rami. After giving off the bronchial rami the vagus joins with the nerve of the opposite side in the formation of the esophageal plexus. From the esophageal plexus small twigs are given off to supply the esophagus. These twigs remove almost all of the remaining medullated fibers, so that the nerve as it enters the abdomen is composed almost entirely of non-medullated fibers.

Figure 18 is a low-power drawing of the anterior half of the esophageal plexus. The fascicles of this plexus have been dissected off from the esophagus, drawn close together, and stained with osmic acid. There are very few medullated fibers in these fascicles. A high-power drawing of fascicle *a* is seen in figure 19. The few medullated fibers seen here are widely separated and of very small size. Pyridine-silver preparations show that these fascicles are almost entirely formed of solidly packed, fine, non-medullated axons. In figure 16 one sees a high-power drawing from one of these preparations. In this field there are six medullated fibers which are almost lost among the great number of non-medullated axons.

As the nerve passes through the diaphragm it contains very few medullated fibers (fig. 20). It will be noted that figures 17 and 20 were taken from preparations of the cat's vagus. The thoracic vagus in the cat, then, has the same structure as that in the dog. The two figures represent the same nerve, the one taken above the level of the pulmonary rami and the other as the nerve pierces the diaphragm. A comparison of these two figures shows how great is the decrease in the number of medullated fibers as the nerve passes through the thorax. Those which have disappeared have either left the vagus through the pulmonary rami and fine branches to the esophagus, or have lost their sheaths in their downward course.

Gaskell also noted this decrease in the number of medullated fibers and concluded that the vagus as it pierced the diaphragm was composed chiefly of non-medullated fibers.

THE SOURCE OF THE NON-MEDULLATED FIBERS OF THE VAGUS

This great predominance of non-medullated over medullated fibers in the vagus as it passes through the diaphragm seems to us of considerable importance in connection with the interpretation of these fibers in the vagus. It seems almost certain that the vagus at this level contains more efferent pre-ganglionic fibers than would be accounted for by the few medullated fibers which it contains; and it does not seem at all probable that all of the non-medullated fibers are afferent in function, otherwise the abdominal vagus would be almost wholly an afferent nerve. It would therefore seem probable that there are efferent non-medullated fibers in the thoracic and abdominal portions of the vagus nerve.

It will be remembered that the proportion of non-medullated fibers increased steadily in following the vagus downward in the neck and thorax. This would fit in well with the theory advanced by Langley and summarized in a preceding paragraph of this paper that some of the pre-ganglionic fibers of the vagus lost their sheaths within the vagus trunk. Whether any of the non-medullated fibers in the roots of the vagus are pre-ganglionic fibers or not remains an open question.

Reasoning from the analogy of the spinal nerves, where the non-medullated fibers of the dorsal roots are probably all afferent in function and arise from the small cells of the spinal ganglia (Ranson '12), it seems probable that many, if not all, of the non-medullated fibers in the roots of the vagus arise from cells in the jugular and nodose ganglia and are afferent in function. It seems probable, therefore, that both afferent and efferent non-medullated fibers are found in the vagus nerve.

SUMMARY

1. The various rootlets of the vagus and accessory nerves differ markedly in structure. The *spinal root of the accessory* is composed almost entirely of large medullated fibers with a very few small ones. The *bulbar rootlets of the accessory* are composed of large and small medullated fibers, with the small fibers predomi-

nating. These rootlets contain few, if any, non-medullated fibers. The *rootlets of the vagus* are of two kinds. Those of Type I, probably efferent in function, are composed of many fine and fewer coarse medullated fibers. The medullated fibers are evenly distributed through these rootlets and there are few, if any, non-medullated fibers. The vagus rootlets of Type II, probably afferent in function, contain large and medium-sized medullated fibers and fewer small ones. The medullated fibers are widely separated by enormous numbers of fine, non-medullated axons.

2. At the level of the upper part of the jugular ganglion the vagus and accessory nerves are fused into a common trunk in which it is possible to distinguish three areas derived respectively from the spinal root of the accessory, the bulbar roots of the accessory, and the roots of the vagus. Each area presents the same histological characteristics as the corresponding roots, except that the fibers from the two types of vagus rootlets are now intimately mingled.

3. Below the level of the jugular ganglion the spinal part of the accessory, which has maintained its independence throughout the common vagus-accessory trunk, now leaves it as the external branch of the accessory nerve.

4. The bulbar fibers of the accessory become intimately mingled with the vagus fibers at or above the level of the nodose ganglion. The so-called internal branch of the accessory does not exist as a separate nerve, but is only a fascicle of the common vagus-accessory trunk.

5. While the vagus and sympathetic nerves are intimately associated in the neck, it is clear that no considerable part of the non-medullated fibers of the vagus are of sympathetic origin.

6. The pharyngeal branch is composed for the most part of large medullated fibers, but also contains a considerable number of medium and small-sized medullated fibers. It contains few, if any, non-medullated axons.

7. The superior laryngeal branch contains large, medium and small-sized medullated fibers, with the medium and small ones predominating. It contains non-medullated fibers in consider-

able numbers; but these are much less numerous than in the vagus trunk.

8. The recurrent nerve contains an area of large medullated fibers destined for the larynx, and an area of medium and small-sized medullated fibers which are given off in its esophageal, tracheal and cardiac branches.

9. The pharyngeal, superior laryngeal and recurrent nerves take out from the vagus trunk almost all of its large medullated fibers, so that the vast majority of the medullated fibers in the thoracic vagus are either small or medium-sized. These are widely separated by non-medullated fibers.

10. The non-medullated fibers are present in much greater proportion in the thoracic portion of the vagus than in the upper part of the nerve. This is to be accounted for by the fact that a large number of medullated fibers have been taken away by the cervical branches, while practically all of the non-medullated fibers are carried down into the thoracic vagus. This increase in the proportion of non-medullated fibers in the lower part of the nerve is probably also in part due to pre-ganglionic fibers losing their myelin sheaths in their downward course.

11. Most of the medullated fibers in the thoracic vagus leave it through the bronchial and esophageal branches, so that the vagus as it passes through the diaphragm may properly be called a non-medullated nerve. It is composed almost entirely of non-medullated axons and contains only a few scattered medullated fibers.

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REGENERATION OF MEDULLATED NERVES IN THE ABSENCE OF EMBRYONIC NERVE FIBERS, FOLLOWING EXPERIMENTAL NON- TRAUMATIC DEGENERATION

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THIRTY-TWO FIGURES

INTRODUCTION

The present study is based upon experiments in which degeneration and regeneration of medullated nerve fibers were brought about under new experimental conditions. The results obtained relate, for the most part, to phases of the subject upon which the evidence has heretofore been incomplete. In this investigation, an experimental obstacle which has been responsible for the strikingly contrary observations between the supporters of auto-regeneration on the one hand and the advocates of an out-growth of the axis cylinder on the other, has been entirely avoided. I refer to an ingrowth of foreign nerve fibers through the scar tissue into a regenerating medullated nerve. This obstacle was avoided by inducing degeneration in the peripheral medullated nerves of the domestic fowl by a prolonged exclusive feeding of polished rice, and subsequent regeneration by a return to an adequate nutritive diet.

In 1897 Eijkman first described 'polyneuritis' in fowls which had been kept for three or four weeks on an exclusive diet of polished rice. This has since been confirmed by numerous other investigators and Frazer and Stanton ('11) have noted and illustrated 'Wallerian degeneration' in the nervus ischiadicus of the domestic fowl which developed paralysis on a polished rice¹ diet.

¹ White rice, polished rice or decorticated rice is the clear white table rice of commerce. It is rice, which, after having the husk taken off, is further sub-

In another place² I have described more in detail the changes occurring in the nervous system of such fowls. Here it was also pointed out, in agreement with Frazer and Stanton and others, that "The neuritis produced in fowls by a prolonged diet of polished rice is, so far as the best evidence indicates, a neuritis due to a deficiency of some food constituent or constituents necessary for the maintenance of the metabolic and functional activity of the nervous system."³

In the paralysis of fowls brought about by an exclusive diet of polished rice the medullated fibers of the sciatic undergo a rapid degeneration. This degeneration, however, is much slower than that produced as a result of transection of the nerve. Moreover, for the rice-fed fowls, the following conditions obtain: In

jected to a process of 'milling' or polishing. "In this process the fruit wall or pericarp, the layers subjacent to it (the subpericarpal layers) as well as the embryo are removed," Frazer and Stanton ('11). These authors give the following as the average composition of polished and unpolished rice:

	PROTEIN	FAT	CARBO- HYDRATE	ASH	MOISTURE
	per cent	per cent	per cent	per cent	per cent
Polished rice.....	7.7	0.25	77.23	0.25	14.3
Unpolished rice.....	9.0	1.65	75.52	1.08	12.75

Unpolished rice or red rice is rice which has not been subjected to the polishing process, and which as a consequence still has the pericarp, subpericarpal layers and the embryo. Fowls fed exclusively upon unpolished rice for long periods never develop neuritis as when fed exclusively upon polished rice. Further, neuritis in fowls as the result of an exclusive rice diet can most frequently be cured by placing the fowl on an exclusive diet of unpolished or red rice. There are several qualities of white rice, which, aside from the quality of the grain, are denoted by the amount of polishing to which the rice has been subjected. As might be expected the most highly polished grade is the most effective in producing paralysis in the fowl.

² Edward B. Vedder and Elbert Clark. A study of polyneuritis gallinarum. Philippine Journal of Science, vol. 7, no. 5, Sec. B, p. 423.

³ Richard P. Strong and B. C. Crowell have produced experimentally in man a similar neuritis by the prolonged feeding of a diet of which polished rice formed by far the main constituent. In one case which resulted fatally, the peripheral nerves showed marked degeneration (The etiology of beri-beri. Phil. Jour. Science, B, vol. 7, p. 271). John M. Little has also described beri-beri in man resulting from an almost exclusive diet of white bread (Beri-beri caused by fine white flour. Jour. Amer. Med. Assoc., vol. 58, p. 2029).

degeneration the fibers are intact and all traumatic and inflammatory effect produced by cutting the tissues and the nerve or of tying the latter are obviated; the process of degeneration can be stopped at almost any stage or greatly prolonged, and several stages of degeneration are to be observed in different fibers of the same nerve. In regeneration, the possibility of an ingrowth of fibers from other nerves into the regenerating nerve under observation is obviated and recovery of the animal can be accomplished after any stage of degeneration of the peripheral nerves. And lastly, the slowness of the cycle of degeneration and recovery, makes it possible to draw a sharper distinction between the process of degeneration and regeneration in medullated nerve fibers. At the present time these experimental conditions are especially desirable.

PARALYSIS IN FOWLS RESULTING FROM AN EXCLUSIVE DIET OF POLISHED RICE

A typical case of a fowl which has become unable to walk after a diet of polished rice is found in that of No. 54 whose history is as follows: No. 54, brown hen, fed polished rice since February 7, 1912; 25 days later, on March 3, the first definite signs of unsteadiness in the legs were noted. March 4, the bird was found balanced on its 'haunches,' was scarcely able to rise and could not take more than two or three haphazard steps without tumbling over in a heap. The diet was changed to a 'regenerative' diet consisting of whole grain, meat scraps, bread, grass, etc. March 14, could stand up very unsteadily for a few seconds but was scarcely able to take a step. March 20, same—never stood up nor attempted to walk unless forced and assisted in this. April 3, good general appearance but was scarcely able to walk. April 10, improved, but walked with much difficulty. May 3, apparently entirely recovered within the last few days.

It should be noted that many fowls on the polished rice diet lose complete control of the lower part of the legs. Others show wing drop, droopiness of the head and inability to swallow.

Still others show complete collapse.⁴ The greatest variety of symptoms are manifested by various birds, but loss of control of the legs is the most frequent. Fowls showing the latter symptom, with otherwise fair to good general condition, were the ones selected for this study; nerves of these show a more pronounced degeneration, and recovery in this class of fowls is more easily accomplished. Twenty to thirty days on the white rice diet is the usual length of time before symptoms of neuritis are manifested. Some birds resist for 35 or 40 days, and two fowls⁵ that were receiving a small quantity of calcium lactate with the rice did not 'come down' till the fifty-first and sixtieth day respectively. Nitrogenous and fatty foodstuffs in very small amounts added to the rice also greatly defer the development of the neuritis. For more complete data on this interesting affection and for feeding experiments, reference should be made to the recent article by Vedder and Clark ('12).

DEGENERATION

A few remarks should be made at this point concerning the nature and extent of the degeneration in the medullated fibers of the sciatic nerve in fowls of the class under consideration. In the nerves of 60 chickens, which had been fed 20 days or more on an exclusive diet of polished rice, degeneration in the fibers of the sciatic nerve was observed by the aid of the Marchi method in every case regardless of what symptoms were manifested by the fowl before death. Many of these were confirmed by the Weigert method for staining the myelin sheath. Several fowls fed as long as 35 to 40 days showed no signs of weakness in the legs but well marked nerve degeneration. The nerves from each of twelve fowls fed from 7 to 22 days consecutively

⁴ Several workers have observed that fowls occasionally do not lose weight on the polished rice diet. Frazer and Stanton ('11) who have kept very complete records report several fowls which kept their weight up for as long as 35 days. Other fowls even showed a gain in weight.

⁵ Courtesy of Dr. R. B. Gibson of the Department of Physiology; from experiments being conducted by him to study the influence of an addition of various salt mixtures to the white rice on the production of this affection, to be reported shortly.

with no leg weakness showed, by the same methods, myelin degeneration in their fibers. It was a constant observation that different fibers of a given nerve present the greatest variation in the degree of their degeneration. In two fowls killed after feeding 7 days on white rice, small areas of blackening after treatment by the Marchi method were observed in approximately one-third of the fibers of the sciatic. These areas very seldom involved the entire diameter of the individual fiber at any one point and the great majority ranged from 1 to 8 microns in diameter (fig. 32).

In the sciatic nerve of those fowls fed for a longer time and which developed a typical paralysis in the legs, every fiber showed larger areas of blackening. Advanced degeneration was found in from 10 per cent to 20 per cent of the fibers. The change shown by these latter fibers presents an identical picture of degeneration with that in medullated fibers of a mammalian nerve 10 to 14 days after section, but for the nuclei of the neurilemma sheath. The nuclei of the neurilemma sheath have undergone little or no multiplication. This will be again referred to and more fully discussed in the consideration of the "embryonic nerve fiber." By both the Marchi method and the Weigert method for the medullary sheath, the change of the medullary sheath substance into fatty globules and droplets appears complete in some fibers. As I have pointed out in a previous paper ('12),

Fibers showing advanced degeneration are marked by accumulation of degenerated myelin in large globules and droplets, a swelling and bulging of the nerve sheath at these points and a disintegration of the axis cylinder. The largest globules usually appear vesicular and in their center, segments or fragments of the axis cylinder are frequently to be seen. In these larger, and in some of the smaller globules also, the stainable material is found at the periphery and appears laminated. This laminated appearance is very characteristic in Weigert preparations and is the rule in the larger globules. Usually 3 distinct layers are clearly visible, of which the outer is the thickest. Other incomplete layers and fragments are seen centrally.

But for an increase in the number of nuclei of the neurilemma sheath, this description applies with equal exactness to fibers of similar preparations of the peripheral segment of the sciatic nerve of a fowl 7 days after section. Figures 2, 3, 4 and 5

illustrate the marked degeneration described, and figure 6 shows segments of axis cylinders enclosed within large globules.

Howell and Huber ('92), Bethe ('07), Mott, Halliburton and Edmonds ('04), Cajal ('05), Ranson ('12), and others have observed that after section not all medullated fibers degenerate with equal rapidity. As noted, this is particularly true of degeneration in the fibers of the sciatic in fowls on a white rice diet. In the nerves of these fowls, a fiber showing the first indication of degeneration may be found side by side with one in which the neuraxis and medullary sheath are completely broken up (fig. 7).

These few remarks, with the accompanying figures 2 to 7, make it clear that paralysis in the rice-fed fowls presents an experimental condition resulting in degeneration in which almost every stage of the process is to be observed in the same nerve at one and the same time, in which the continuity of the fibers is not disturbed, where reaction to trauma is obviated and where degeneration, though proceeding rather rapidly, is much slower than after section of the nerve.

THE EMBRYONIC NERVE FIBER

Rapid multiplication of the nuclei of the sheath of Schwann, coincident with the change in the medullary sheath and axis cylinder, has been a constant finding with all those observers who have studied degeneration of nerves after section. It was a little surprising to find it not to obtain in the present case which in every other respect resembles Wallerian degeneration and in which the process of degeneration is also rapid. A careful search of both teased and sectioned preparations of nerves, taken at time of paralysis and in which the nuclei were well stained, failed to reveal any marked or definite increase in the number of nuclei of the neurilemma sheath or any structure which could be definitely identified as an embryonic nerve fiber in the degenerated fibers of a single fowl.

An explanation of this marked variation was of course required, and inasmuch as fowls frequently recovered after the most marked paralysis of the legs, it became necessary to determine if regeneration occurs in that 10 to 20 per cent of fibers which show

such marked degeneration after a prolonged diet of white rice. This being the case, it would then be desirable to know whether regeneration could take place in these fibers without going through that stage termed 'embryonic nerve fiber,' 'Bandfiber' or 'protoplasmic band.' For a more complete understanding of the significance of the multiplication of the nuclei of the neurilemma sheath and the embryonic nerve fiber, I sought to determine if degeneration in medullated nerve fibers without multiplication of the nuclei of the neurilemma sheath could be brought about by any other experimental means; and if so, could regeneration be accomplished in these without the embryonic nerve fiber stage? Further, could it be shown that the increase in the number of nuclei usually observed is due to trauma or inflammatory influences or to an infiltration of phagocytes? And lastly, does the embryonic nerve fiber represent a stage of regeneration or degeneration? An answer to these questions would manifestly throw additional light upon the significance of the increase in number of nuclei of the sheath of Schwann and of the embryonic nerve fiber. This proved to be a most attractive phase of the investigation.

Atrophic degeneration without multiplication of the nuclei of the sheath of Schwann has been frequently described in certain chronic pathologic conditions lasting many weeks or months. In the present case, however, where globulation and breaking up of the axis cylinder have been observed as early as the nineteenth day of the white rice diet and where the first evidences of change noted was on the seventh day, the degeneration (taking place in 12 days) can not be said to have anything in common with atrophy.

It soon became evident on histologic examination that regeneration does occur in those fibers in which the neuraxis and medullary sheath have broken up. Having failed to find in the degenerated fibers a single embryonic nerve fiber or any marked or definite increase in the number of nuclei of the neurilemma sheath, I next examined nerves from fowls at various periods during regeneration. With this end in view, fowls were killed after having been kept on a regeneration diet for 4, 11, 13, 14,

16, 19, 21 and 30 days respectively. The fowl which had been kept for 30 days on the nutritive diet showed marked improvement. In addition to nerves from this series, segments of the sciatic, cut out from fowls 48, 56, 59 and 60 days in regeneration respectively, were examined for embryonic nerve fibers and multiplication of the nuclei of the neurilemma sheath. These four fowls had just become able to walk again. In neither teased nor sectioned preparations of the nerves of all these fowls were embryonic nerve fibers to be found. Nor was there observed a more definite indication of multiplication of the nuclei of the neurilemma sheath than was seen in the nerves of those fowls killed at the time paralysis developed. This lack of nuclear multiplication also obtained in nerves of fowls 108, 125, 171 and 275 days in regeneration.

Attempts to produce by other means a degeneration in the medullated fibers without a multiplication of the nuclei of the sheath of Schwann were not successful. Freezing a small portion of the sciatic with carbon dioxide snow, treating a portion with chloroform vapor, and injecting a drop or two of chloroform into the nerve, when successful in producing degeneration, resulted also in the typical and rapid increase in the nuclei of the neurilemma sheath as has been constantly described after section or ligature of the nerve. Less violent means were then adopted in that rubber bands, tight enough to cut off the circulation but not tight enough to do mechanical injury to the nerve were placed around the thigh of fowls. Loss of control of the leg resulted after 3 or 4 days, both when the bandage was allowed to remain for 24 hours at a time and when it was on and off every few hours over a course of 2 or 3 days. Although by this method the result sought for was not accomplished, yet another observation of equal importance was made. Fowl No. 78, on which the bandage was allowed to remain for 24 hours, experienced considerable oedema of the bandaged leg; this condition progressed, without infection, to dry gangrene of nearly all of that portion peripheral to the bandage by the twenty-eighth day, or approximately 24 days after loss of control of the leg. This, then, represents a nerve in which only retrogressive changes

could have taken place as gangrene early manifested itself--by the twelfth day of the loss of control. Fibers from that portion of the leg affected by gangrene presented an appearance from which they were readily recognized as embryonic nerve fibers (fig. 8). For the most part these were very slender with long nuclei, an appearance which is readily accounted for by the partial desiccation. Many still contained droplets of degenerated myelin. It is difficult to see how the least regenerative reaction could have taken place in this nerve.

As stated above, I have not observed a single instance in which there was a marked increase in the number of nuclei of the sheath of Schwann in those degenerated fibers of fowls which showed paralysis of the legs after 20 to 30 days on polished rice. In fibers showing the most advanced degeneration, that is, marked globulation of myelin and breaking up of axis cylinder, measurements were made between neighboring nuclei of the sheath of Schwann. Among these measurements, there were observed such distances as these between successive nuclei: 368, 386, 477 and 379 microns respectively. The distance between 2 nodes of Ranvier is variously estimated as from 80μ to 900μ according to the diameter of the fiber, and "in the higher vertebrates a single nucleus is found midway between each two nodes"—Huber. Mitosis has not been observed.

Some few fibers, however, were seen in which the nuclei of the sheath of Schwann occurred at intervals frequent enough to be suggestive of a slight increase in their number. This was suggestive enough to make it desirable to examine, for an increase of the number of nuclei of the sheath of Schwann, nerves of fowls with which the onset of paralysis had been deferred by the addition of very small amounts of other foodstuffs to the rice. At this period I was fortunate in securing from Dr. R. B. Gibson of the Department of Physiology, a fowl, No. 17, G, which suddenly developed paralysis of the legs after 60 days on a diet of white rice and calcium lactate. This fowl developed a typical case of leg paralysis two days before death. Marchi preparations of the sciatic showed very extensive and advanced myelin degeneration.

Teased preparations from the sciatic of this fowl stained to bring out the nuclei revealed a few fibers of the embryonic nerve fiber type. These were very slender fibers with nuclei at frequent intervals. Protoplasm was scant even around the nuclei, though the structures stained well (fig. 9). Many resembled very closely non-medullated nerves in degeneration described by Ranson ('12). Others were found, however, which contained a larger amount of protoplasm and in which small droplets of degenerated myelin could occasionally be found (fig. 10). This observation clearly shows these to have been derived from medullated nerve fibers. No axis cylinder could be demonstrated by special stains. Another fowl, No. 9, *G*, on a similar diet and with a somewhat similar history developed paralysis of the legs after 51 days. Teased preparations from the sciatic of this fowl, when stained to bring out the nuclei, also showed embryonic nerve fibers. These were a little more numerous than in No. 17, *G*, were larger and contained more protoplasm. Protoplasmic granules were seen in the immediate neighborhood of the nucleus and droplets of degenerated myelin were of rather frequent occurrence. Many of the fibers bore an exact resemblance to the protoplasmic bands to be seen in medullated nerves after section. Figure 11, which is from a teased preparation of the sciatic nerve of No. 9, *G*, illustrates this resemblance and shows beyond question their identity with the embryonic nerve fibers of a sectioned nerve.

Fowl No. 57, which came down with severe paralysis and prostration after 23 days on a diet of polished rice and which made a most difficult recovery, was killed 1 year and 14 days after being placed on a regeneration diet and approximately 10 months after all symptoms of paralysis had disappeared. Teased preparations, stained as above, showed among several thousand fibers one very frail fiber with nuclei at frequent intervals. The fiber appeared scarcely more than a strand of connective tissue with well staining spindle-shaped nuclei along its course. This was probably an embryonic nerve fiber. Previous observations had convinced me that regeneration of the axis cylinder fails to take place in a very small percentage of those fibers which

have shown degenerative changes. This slender fiber with frequent nuclei, then, might easily represent a later stage or atrophy of a fiber which failed of regeneration.

To summarize briefly: Neither embryonic nerve fibers nor a marked multiplication of the nuclei of the neurilemma sheath have been found in degenerating fibers of fowls showing paralysis after 20 to 30 days on polished rice: they have not been observed (with one possible exception) in regenerating fibers of fowls recovering from this paralysis: embryonic nerve fibers containing droplets of degenerated myelin were found in the nerves of fowls fed for 51 and 60 days on a polished rice diet, and embryonic nerve fibers entirely replaced the nervous elements in the sciatic of a fowl whose leg was undergoing progressive necrosis.

This chain of evidence seems complete and can leave little doubt as to the significance of the so-called embryonic nerve fiber, Band-faser or protoplasmic band. In each instance except the last we have the embryonic nerve fiber occurring in a nerve which is undergoing progressive retrogression. In fowl No. 78 the degeneration was due to mechanical causes. In No. 17, G, and No. 9, G, those few fibers which always showed marked degeneration by the thirtieth day, regardless of whether symptoms are manifested or not, had had time for a more advanced degeneration than those fibers of fowls killed at an earlier date. In either case, it was impossible for regeneration to be taking place.

Thus the conclusion that the embryonic nerve fiber represents a late stage of degeneration is a logical one. Degeneration as used above is meant to imply a retrogressive change in the myelin sheath and axis cylinder. It is to be noted that multiplication of the nuclei of the neurilemma sheath and the resulting embryonic nerve fiber appear only after degeneration in the axis cylinder and medullary sheath has advanced to a late stage. As Van Gehuchten points out, the sequence of events in the formation of the embryonic nerve fiber with cytoplasm and cytoplasmic granules around the nuclei must be considered evidence of protoplasmic activity and in themselves bear a close resemblance to regenerative processes. This, however, does not necessarily imply an attempt on the part of the structure at formation of a new

medullary sheath or axis cylinder. Indeed, Mott, Halliburton and Edmonds ('04) in regeneration in nerves after section, find that the new medullary sheath, as well as the axis cylinder, progresses from the point of union of central and peripheral stumps. I will suggest that the multiplication of the nuclei of the neurilemma sheath and the formation of the embryonic nerve fiber in the rice-fed fowls is comparable to the proliferation of connective tissue in organs undergoing atrophy or degeneration from other causes. In the fowls degeneration of the nerve fibers is slow and it is probable that the stimulus is not sufficient to bring about a multiplication of the neurilemma nuclei until very late. On the other hand, the trauma occasioned by transection of the nerve introduces a violent reaction and multiplication of the nuclei of the neurilemma sheath rapidly ensues.

INFILTRATION OF PHAGOCYTES AND ABSORPTION OF THE DEGENERATED MYELIN

Stroebe ('93), Mott, Halliburton and Edmonds ('04), Nageotte ('11) and other have described an infiltration of phagocytic wandering cells into medullated fibers undergoing myelin degeneration. Nageotte ('11) claims it as a constant occurrence and sees in their presence a means of removal of the degenerated myelin. According to Nageotte ('11), these foreign elements

constitute the agents of greatest activity in the resorption of the degenerated myelin. This does not signify that the syncytium of Schwann remains inert; it can, indeed, resorb the myelin, and it is probable that, in the fine fibers, it accomplishes the work of phagocytosis of the myelin without the aid of the foreign elements. In the large fibers, at the end of the third day, one sees in the perinuclear protoplasmic mass special granulations which result from the disintegration of the myelin; but, in these fibers the larger part of the myelin becomes the prey of the leucocytes. It is probable that the leucocytes emigrate once their work is accomplished.

In nerves of fowls with marked leg paralysis after about 30 days on polished rice, I have sometimes seen fibers in degeneration in which the appearance of infiltration by wandering cells is very striking. These cells were all very small and, after Müller fixation, scarcely anything but the nucleus was to be seen in

hematoxylin and carmine preparations. In an occasional fiber they were quite numerous, though uniformly absent from those fibers showing the larger globules of degenerated myelin with enclosed segments of the axis cylinder. Figure 12 shows the most marked instance of infiltration observed in this series. Here it is clearly evident that not more than one nucleus belongs to the sheath of Schwann. These nuclei have not been observed in fibers from fowls which developed paralysis after 51 days or more on polished rice. Their presence in some fibers, however, is sufficient to show that trauma is not alone responsible for their presence.

I have not been able to determine the significance of these wandering cells or their fate. That their participation in the removal of the degenerated myelin is improbable and at most can be only extremely slight, is clearly manifested by the persistence of degenerated myelin seen in fibers of Marchi preparations from fowls which had been kept a long time on a regeneration diet and for several months after all symptoms of paralysis had disappeared.

In nerves from these fowls, both small droplets and large globules of degenerated myelin still obtained, and indeed in some fibers the picture resembled very closely that of degeneration in medullated nerves of fowls but recently affected by paralysis. There seemed to have been some little absorption of degenerated myelin in the nerves of those birds kept for 4 months in regeneration, yet globulation was just as marked and the globules were equally large. The globules of degenerated myelin in the fibers of fowls dead just after paralysis appeared dense, while in the fibers of fowls 4 months in regeneration the globules frequently appeared honey-combed or somewhat reticulated. The droplets of degenerated myelin were uniformly smaller in the latter. Fowl No. 38 (leg paralysis after 22 days on polished rice, recovery apparently complete after 59 days on regeneration diet) was killed on the 108th day of regeneration or 49 days after all symptoms of neuritis had disappeared. Every fiber of the sciatic nerve of this fowl contained droplets or globules of myelin and many of the larger globules just described. Even

the swelling of the fiber and out-bulging of the sheath at the large globules obtained and the larger globules were still vesicular. Figure 13 is a photomicrograph of a Marchi preparation of the sciatic nerve from this fowl. Droplets of degenerated myelin are clearly visible in every fiber and the larger globules occur at frequent intervals. Figure 14 is a similar picture of the sciatic of No. 54, 171 days in regeneration. The droplets and globules of degenerated myelin are equally definite here.

Degenerated myelin was found to persist in the sciatic nerves of a fowl 10 months after all signs of paralysis had disappeared and 1 year and 14 days after regeneration diet was started. Figure 15 is taken from a Marchi preparation of the sciatic nerve of No. 57, whose recovery history was as is just indicated. The largest globules have completely disappeared or more probably have considerably decreased in size. The smaller droplets are also decidedly less numerous and the total amount of degenerated myelin is comparatively small.

In view of the finding that the sensory nerves usually show an apparent recovery before the motor, it was thought worth while to compare the sensory and motor portions of the sciatic nerve of No. 57 (1 year and 14 days in regeneration). Accordingly the motor and sensory portions of one of the larger roots were separated for some distance peripheral to the dorsal root ganglion, each stained by the Marchi method and teased. No difference whatever could be distinguished between the two. It was quite impossible to tell one from the other (compare fig. 15 and fig. 16).

It would thus seem that, in the present case at least, phagocytes are in no way concerned in the removal of degenerated myelin. Phagocytosis is clearly excluded inasmuch as the disappearance of myelin is so extremely slow and since wandering cells are as a rule not found in degenerating nerve fibers of the fowls under consideration.

Just why degenerated myelin should persist so long in nerve fibers of these fowls and disappear so quickly from those nerves which have been sectioned or ligated or even from No. 78 of my series, whose leg had been bandaged for 24 hours, is not clear. The above observations would seem to exclude removal

by wandering cells which are common to both. An explanation might be sought in the multiplication of the nuclei of the sheath of Schwann and the resulting embryonic nerve fiber, which are such marked characteristics of degeneration in the latter, while being uniformly absent from the nerves of fowls showing an early paralysis after a white rice diet. This is rendered still more probable when it is remembered that in those fowls in which paralysis was deferred—No. 17, G, and No. 9, G—typical embryonic nerve fibers were present and within these fibers degenerated myelin occurred in only very small amounts or was entirely absent. These two fowls differ from those showing paralysis at an earlier date in that in the former degeneration in a certain proportion of the fibers had progressed to a later stage than with those coming down at an early period. Were it possible to keep the fowl alive on the white rice diet till all fibers were given opportunity to undergo advanced degeneration, I have no doubt but that after regeneration in the same animal the medullated nerve fibers would be quite devoid of even droplets of degenerated myelin.

In those fowls that have recovered from paralysis after 20 to 30 days on polished rice, the whole chain of evidence convinces me that here degeneration is interrupted in the middle, as it were; and regeneration is accomplished or superimposed without passing through the later stages of degeneration. This being the case, we must attribute the rapid removal of degenerated myelin in sectioned nerves to the activity of the new nuclei of the sheath of Schwann and the embryonic nerve fiber. This assumption is further borne out by the well-known observations that in medullated fibers of the central nervous system in degeneration the globules of degenerated myelin persist for a very long time. Halliburton ('07), in speaking of this point, says: "In situations like the central nervous system where the neurilemma is non-existent, not only is the removal of degenerated myelin a very slow process, but as is well known, regeneration does not occur." Schröder ('08) points out, in speaking of degeneration in medullated fibers of the cord: "Noch nach einem Jahr sind grobe Schollen sowie namentlich feine Tröpfchen zu

finden." This is a constant finding in fibers of the cord unless special precautions are taken to produce marked inflammation of the affected area, such as by infection. Then the degenerated myelin is removed somewhat more rapidly, though even here not so rapidly as in the peripheral nerves after section. With pronounced inflammation other factors are introduced which would readily account for the more rapid removal of the degenerated myelin; as they have no bearing on the question they need not be considered here. Now, medullated fibers of the cord differ histologically from medullated fibers of the peripheral nerves in that the former do not possess a neurilemma. After section Wallerian degeneration of the one differs from that of the other only in that the proliferation of the nuclei of the neurilemma sheath and the resulting embryonic nerve fiber are lacking in the fibers of the cord. Infiltrating phagocytes are found in degeneration in both cases.

It is clear then that the rapid multiplication of the nuclei of the neurilemma sheath introduces a factor which is responsible for the rapid removal of the degenerated myelin. It is probable that the protoplasmic activity, represented by the multiplying nuclei and the accumulation of protoplasm around these, is directly concerned with the rapid resorption of the degenerated myelin.

I have no evidence suggestive of a further activity of the embryonic nerve fiber and its nuclei and this phase of the subject was not taken up. A definite zone rich in protoplasm was, however, observed around the nuclei on the embryonic nerve fibers of the sciatic of fowl No. 9, G. In this protoplasm, discrete granules were to be observed in preparations stained with Mallory's phosphomolybdic acid hematoxylin for axis cylinders. These granules have been noted by Reich and others. According to Stroebe ('93), Nageotte ('11) and others they bespeak an activity of the nuclei concerned in the development of a new medullary sheath.

This view in one form or another has been frequently advocated, both as an hypothesis and as an interpretation of the fact that, as pointed out by Stroebe ('93), the protoplasm which

increases in amount with the multiplication of the nuclei and the disappearance of the degenerated myelin in nerves after section, contains numerous small granules and droplets of fatty material. Thus according to Mott, Halliburton and Edmonds ('04), "they (i.e., the nuclei of the neurilemma sheath) multiply, and later appear to share with phagocytes in the removal of the broken up myelin droplets." And Schröder ('08), basing his views upon the microscopic observations of Stroebe and Büngner and Schütte, has the following to say relative to the removal of the myelin clumps:

In this purely degenerative process (i.e., the early clumping of the myelin) early progressive occurrences interpose themselves. The nuclei of the sheath of Schwann begin to proliferate already on the second day, according to Stroebe they attain their maximum increase in number through mitosis at about the eighth day; coincident with the proliferation of the nuclei protoplasm develops, it shoves itself into the breaches and spaces between the myelin clumps, flows around the clumps, then gathers itself together into a single round or oval, demarcated, single-, or many-celled structure, within whose interior the clumps of myelin rapidly diminish themselves to fine granules (according to Büngner and Stroebe). In this way arise frequently granular cells with round cell body and a fine latticed protoplasm in whose meshes the granules lie enclosed. Such elements, from about the fourth week on, are to be found in the lymph spaces around the neighboring vessels. Stroebe and Schütte mention, that the genesis of these granular cells has been often incorrectly conceived; with predilection, one has declared them leucocytes or so-called wandering cells and has assumed that they arise from the vessels opened at the point of the primary injury to the nerve, move forward along the nerve sheath and then take up at all places the disintegrated myelin.

REGENERATION

Marked multiplication of the nuclei of the sheath of Schwann at an early stage of degeneration and the resulting embryonic nerve fiber have been constant findings with all those observers who since Waller, have studied degeneration and regeneration of medullated nerves after section. Upon this point there is complete agreement. The interpretation, however, of the significance of the increased number of nuclei and more particularly of the embryonic nerve fiber has given rise, as is well known, to the most heated controversies, often involving personalities. As

a result the most varied experiments have been conducted and a vast amount of evidence on all possible phases of the subject has been presented. Unfortunately, however, the question remains unsettled. Unprejudiced authors of text-books still include both theories.

Two distinct and opposite views relative to regeneration after the embryonic nerve fiber stage are at present current. According to one, most vigorously and ably advocated by Bethe, the embryonic nerve fiber is capable of producing per se a new medullary sheath and new axis cylinder, which, later making connection with the central stump, results in a regenerated and functioning medullated nerve fiber; in young animals at least, the regenerated fiber is capable of conducting impulses regardless of whether or not connection with the central stump is established. The supporters of the contrary theory claim that a new axis cylinder for the peripheral stump is attained only by a down growth of axis cylinders from the central stump. While there is some difference of opinion as to minor points, this is the main contention of those who advocate the 'outgrowth' theory. The sequence of events as interpreted by the adherents of this theory is briefly set forth by Halliburton ('07) as follows:

From the microscopic study of the distal portions of divided nerve trunks, we arrived at the conclusion that the activity of the neurilemmal cells has some relation to the development of new nerve fibers. At an early stage in degeneration their nuclei multiply; later they participate with phagocytes in the removal of the broken up myelin droplets; subsequently they elongate and, becoming connected end to end, lead to the formation of what some have termed "embryonic" nerve fibers. We arrived finally at the conclusion similar to that which Howell and Huber reached fifteen years ago, that, although the peripheral structures are active in preparing the scaffolding, the axis cylinder which is the essential portion of the nerve fiber has an exclusively central origin.

Stroebe ('93), Huber ('95), Cajal ('05), Marienescio ('06), Ranson ('12) and others have described and illustrated microscopic preparations of medullated nerves in regeneration after section which seem to show beyond a doubt that outgrowth of the axis cylinder from the central stump does take place. On the other hand Bethe ('02), who bases his opinion mostly upon physio-

logical grounds, has met the objections of his opponents in a most creditable manner by repeated experiments and new evidence which appear irrefutable. Langley and Anderson ('04), Lugaro ('05) and Mott, Halliburton and Edmonds ('04) have repeated many of Bethe's experiments but oppose rather than support him in his contention for auto-regeneration in the peripheral stump. Langley and Anderson ('04) admit that the peripheral stump, after a sufficient length of time has elapsed, may be found capable of conducting impulses even when union with the central stump is successfully prevented. They explain this apparent auto-regeneration by an ingrowth of nerve fibers from the surrounding tissues. In every case where the peripheral stump became capable of conducting impulses, strong evidence was obtained to show that an ingrowth of fibers into it from other neighboring nerves had taken place. Mott, Halliburton and Edmonds ('04) have confirmed this finding and have found that in a piece of medullated nerve transplanted in the same animal in such a manner that an ingrowth of fibers from other nerves is prevented, regeneration fails to take place. They have further shown that in a regenerating nerve the medullary sheath "appears earliest at situations near the point where the ends of a nerve have been joined together, and reaches the distal portions later." Bethe ('07) has again repeated these experiments but can find no reason to abandon his former strong conviction that auto-regeneration takes place in the peripheral portion of a divided medullary nerve. The experimental work of Ballance and Purves Stewart ('01) lead them to declare for auto-regeneration, and Van Gehuchten ('04) has confirmed Bethe's results. Wilson ('09) after repeating some of Bethe's work, draws no specific conclusion regarding auto-regeneration of divided medullary nerves.

In short the main contention of the advocates of auto-regeneration hangs on whether there may be no ingrowth of foreign fibers from neighboring nerves into the peripheral portion of a divided nerve. This at bottom is the point in dispute between a class of able workers who hold to auto-regeneration and a group of equally acute observers who advocate the outgrowth of the axis cylinder from the central stump. So long as so cap-

able investigators are unable to obtain the same result in a given experiment, just so long will our theories on regeneration of divided medullated nerves remain at variance. One cannot help but suspect, however, that on account of this very difference in results and the very heated controversy on the subject, regeneration of medullated nerves after section has received more attention and investigation than the comparative importance of the subject would warrant.

It was with no desire to engage in such a discussion that the present work was begun, and the new points brought out by it in regard to degeneration will greatly outweigh the observations on regeneration. On the other hand, however, I considered the controversy of others no excuse for avoiding the subject when a promising experiment was thrown at my door.

As stated above, in fowls fed for a long time on polished rice, there results an acute degeneration in the medullated nerve fibers resulting in a breaking up of the myelin into large globules and droplets and a segmentation of the axis cylinder. This change takes place in from 12 to 18 days and bears the closest resemblance to degeneration in medullated nerve fibers after section.

Clearly then, if it could be shown that the process of degeneration in the nerve fibers of the rice-fed fowls was identical with degeneration after section and if regeneration takes place in the former, then regeneration of medullated fibers was accomplished without the possibility of an ingrowth of fibers from other nerves and the main ground for a difference of opinion on auto-regeneration was obviated. If, on the other hand, degeneration in the two is not identical nor comparable, it would still be of interest to know if regeneration in the rice-fed fowls is or is not concerned with the so-called 'embryonic nerve fiber,' '*Band-faser*' or 'protoplasmic band;' if auto-regeneration obtains; or if the new axis cylinder results from an outgrowth of the central connection. Whatever the result arrived at, the facts collected from this new type of experiment would add evidence to one side or the other and argue for or against auto-regeneration.

While there could be found no other microscopic differences in degeneration in medullated fibers after section and in medul-

lated fibers of the rice-fed fowls at the time of paralysis, multiplication of the nuclei of the neurilemma sheath and the embryonic nerve fibers were conspicuous by their absence from the nerves of the latter fowls. Could regeneration be accomplished in such nerves, without a multiplication of the neurilemma nuclei, the significance of the embryonic nerve fiber would be minimized.

With the above questions in mind, regeneration was studied in the sciatic nerve of fowls which came down in 20 to 30 days with marked leg paralysis and which were, from time to time, placed on a regeneration diet. To recapitulate briefly certain observations noted above; in fowls of this class, those medullated fibers, presenting at the time of paralysis the most marked degeneration showed, at most, only a doubtful increase in the number of nuclei of the neurilemma sheath and no embryonic nerve fibers. Nerves of fowls killed after feeding from 4 days to 2 months on the regeneration diet never showed the marked multiplication of the nuclei of the neurilemma sheath or the embryonic nerve fiber, as has been constantly described for mammalian nerves after section, and as was found also in the nerves of fowls in which I had transected the sciatic. Segments of the sciatic of one side cut out and compared with the sciatic of the other side at a later date, showed that in none of these cases had the looked for change in this respect taken place. In other nerves after 108, 125, 171 and 275 days in regeneration, the nuclei of the sheath of Schwann could not be said to be more numerous than in preparations taken at the time paralysis developed, and the embryonic nerve fiber could not be found. The close resemblance of the Marchi preparation from fowls 108 days and 171 days on a regeneration diet to those from fowls at the time of paralysis has also been pointed out and is clearly seen by comparing figures 2, 3, 4 and 5 with figures 13 and 14. Numerous large globules and small droplets of degenerated myelin are to be seen in each.

In view of this condition of the myelin, the uniform absence of embryonic nerve fibers, and the fact that a great majority of fibers of the sciatic do not show a breaking up of the axis cylinder at the time of paralysis, it was at first suspected that regen-

eration failed to take place in those medullated fibers showing the most marked degeneration; and that in its recovery the fowl gradually learned to do without these fibers. Further study convinced me, however, that this was not the case, and that those fibers which had shown such marked degeneration finally attained a new axis cylinder. This conclusion became evident after a close study of the axis cylinder in those fowls which had recovered from paralysis.

Mallory's phosphomolybdic acid hematoxylin, carmine, Cajal's new silver impregnation method for axis cylinders, and Ranson's modification of the last were used for staining the axis cylinder. The preliminary treatment (i.e., hardening in absolute alcohol) called for in the silver methods produced such shrinkage of the fibers that it was often impossible to obtain satisfactory teased preparations. The degenerated myelin was also dissolved out to such an extent, that together with the shrinkage, relations were so distorted that it was usually difficult to distinguish an old from a new axis cylinder and to tell the relation of the latter to the globules of myelin. With the first two methods it was possible to stain a preparation in bulk, clear and tease out without passing through the higher alcohols or xylol. The globules of degenerated myelin were thus preserved. With the phosphomolybdic acid hematoxylin, which gave beautiful results after proper fixation with Müller's fluid, the procedure was as follows: Fix in Müller's fluid (small pieces as fresh as possible), wash 1 to 2 hours in running water, partially tease out a segment of the nerve not more than 1 mm. long to permit rapid infiltration of the stain, place in a ripened solution of Mallory's phosphomolybdic acid hematoxylin 20 minutes to over night, blot off excess of stain and differentiate in 50 per cent alcohol made slightly alkaline with ammonia, pass through two or three changes of 91 per cent alcohol, clear in origanum oil, tease, blot, and mount in xylol balsam. Carmine preparations were prepared in a similar manner but differentiated in weak alcohol without the ammonia. For longitudinal and cross sections, pieces of nerve after washing were rapidly dehydrated and cleared and mounted in paraffin and stained as indicated. Tissues were also fixed in

alcohol and in Bensley's chrom-sublimate solution for carmine staining.

It had been previously shown by me ('12), that, in degeneration in the rice-fed fowls at time of paralysis, there is just as large a percentage of fibers showing advanced degeneration in the sciatic as in its peripheral rami. In regeneration, then, there should be, in the earlier stages, a greater percentage of fibers containing no axis cylinder in the peripheral branches than in the sciatic itself; provided of course, the new axis cylinder is the result of an outgrowth. To determine this, segments of the sciatic and its peripheral rami were cut out in the above series of regenerating fowls for comparison with each other and with the sciatic of the other side at a later date.

The first indication of a regeneration of the axis cylinder was obtained from fowl No. 54 which developed marked leg paralysis on March 4, 1912. The animal was placed on the regeneration diet on March 7. By May 3, all signs of neuritis had disappeared and complete use of the legs had been regained. On this day, on the left side, segments of the nerve were cut out from the upper part of the thigh, and from near the foot. An examination of transverse sections of these two pieces revealed a greater proportion of medullated fibers devoid of axis cylinder in the peripheral segment than in the segment from the thigh. Out of 742 fibers in the peripheral portion, the axis cylinder was wanting in 11; in 1365 fibers (counted at random) in the proximal portion, the axis cylinder was wanting in 9. The fowl was killed 114 days later, on August 25. Transverse sections of the sciatic of the opposite side on this date, revealed an axis cylinder in practically every fiber. In 5788 fibers the axis cylinder was wanting in 8. Similar data were obtained from the nerves of other rice-fed fowls, No. 52, No. 57 and No. 64. However, as degenerated fibers may not be evenly distributed throughout the sciatic, one peripheral nerve may contain a larger percentage of degenerated fibers than its neighbor and relatively more than the sciatic of which it is a branch. As misleading results might thus be obtained, this method of comparison was not prosecuted further.

These findings having indicated that, under favorable conditions, regeneration of the axis cylinder may take place in the degenerated nerves of the rice-fed fowls, more definite evidence of regeneration was sought. This was a difficult and tedious task because it was necessary to show beyond doubt that an axis cylinder in a particular nerve was a new and not an old axis cylinder. Therefore a most careful search was made of sectioned and teased preparations of nerves taken at varying lengths of time after recovery from paralysis.

The sciatic of fowl No. 38—108 days after regeneration diet was begun and 49 days after paralysis had disappeared—was particularly studied because, in addition to being a typical case of peripheral neuritis, the axis cylinders stained exceptionally well and many globules and droplets of myelin were shown by the Marchi stain (fig. 13). In a large fiber containing several large globules of degenerated myelin along its course, a well staining axis cylinder was seen running a tortuous course to one side of the large globules which often occupied almost the entire diameter of the fiber. Two such vesicular globules of degenerated myelin in close proximity to the axis cylinder were seen, which contained in their center segments of a structure which was identified as the old axis cylinder. In figure 17, *m* shows one of the globules in question with its axis cylinder contents. In this figure, *m* clearly represents a single globule of degenerated myelin which has been cut on the tangent by the microtome knife. Part of the old axis cylinder was also probably taken away. The different portions of *a* and *a'*, the new and the old axis cylinders, were not in focus at the same time and the drawing has been constructed, with the aid of a camera lucida outline, to show as nearly as possible in one plane, the relations of these structures. In this figure, *a'* is the exact counterpart of *a* in figure 6 which is readily recognized as broken up axis cylinders within large globules of degenerated myelin. That *a*, figure 17, represents an axis cylinder there can be no doubt.

This observation has been confirmed in other fibers of this same nerve, as well as in the fibers of the sciatic of fowl No. 51, No. 54, and No. 61. In each of these there could be no

doubt about the identity of the structures. When it is remembered that the axis cylinder in advanced degeneration is often quite difficult to stain, it is not surprising that such clear pictures as the above (fig. 17) were not frequently found. The tortuous course of the new axis cylinder around the globules, as well as the different focal levels of fragments of the old, makes it extremely difficult to get a photomicrographic representation which will show both structures in one picture. Figure 18 shows a fragment of the old axis cylinder, *a'*, inclosed within a large globule of degenerated myelin, *m*, in close proximity to the new axis cylinder *a*. In figures 19 and 20 the same condition is shown: *a'*, the fragment of the old axis cylinder; *m*, a globule of degenerated myelin and *a*, the new axis cylinder. It is more frequently the case that the remains of the old axis cylinder are represented only by a mass of granules or fragments enclosed within the globule.

Further confirmation of these observations was readily obtained by a study of cross-sections of the sciatic of these same fowls. In such sections the new axis cylinder could be seen at one side of the myelin globules while segments or fragments of the old were to be seen within the globule. In some fibers the new axis cylinder was a very small structure, 0.5μ or less in diameter, and located quite at the periphery of the sheath. In other fibers it was larger and with its surrounding concentric lamellae occupied an equal proportion of the sheath with the globules of degenerated myelin. Figure 21, fowl No. 54, shows a new axis cylinder, *a*, the old axis cylinder, *a'*, and a globule of degenerated myelin, *m*, in the same cross-section. Figure 22—fowl No. 38—shows a large new axis cylinder, *a*, with its concentric lamellae, *s*, by the side of the old axial tube, *a*; *a'* is also surrounded by concentric lamellae and no large globules of myelin are seen here. It is probable that this is a section of a nerve devoid of myelin globules at this place and in which the axis cylinder has degenerated as a result of its interruption by myelin globules at another level. Figures 23 and 24 are photomicrographs respectively of the same preparations as figures 21 and 22.

All these observations speak strongly for a new axis cylinder in recovery; the greater percentage of fibers with axis cylinders in the sciatic than in its peripheral rami argues also for an outgrowth of the axis cylinder. Further evidence of outgrowth of the axis cylinder was soon obtained. Before I was able to confirm the first observation that a new axis cylinder and segments of the old were to be found in the same fiber at the same time, another fiber was observed in the same preparation, in which growth activity was apparent. This fiber is shown in figures 27 and figures 28 and 29 (photomicrographs) all of the same fiber. It will readily be seen that *b* is an outgrowing branch of the axis cylinder *a*. That *a* is a new axis cylinder is proven by the presence of a fragment of the old axis cylinder, *a'*, (fig. 27 and fig. 29) between the new, *a*, and its branch, *b*. Both axis cylinder and branch stained equally well and much better than the remnants of the old. An end bulb is seen on the tip of the branch. It might be pointed out here that Cajal and Marienescio have observed a similar branching of the outgrowing axis cylinder in medullated nerves after section. These branches, of which there may be one or more to each fiber, often take an abortive course and have been observed to grow in a recurrent direction up the central stump. Whether, in the present case, *b* is an outgrowth from *a* or whether at an earlier stage both were outgrowing buds of approximately equal size is purely a matter of speculation. It should be added that in cross-sections old sheaths were observed which contained two axis cylinders of approximately equal size, each surrounded by a secondary sheath of its own. But more frequently one is much larger and occupies a more central position than the smaller which may be located quite near the periphery of the sheath. Figures 25 and 26 from the sciatic of fowl No. 54, show in transverse section two axis cylinders in the same nerve sheath, and apparently in the same portion of the fiber that was formerly occupied by the old axis cylinder. Whether the zone around each represents a newly acquired myelin sheath I have not determined. Nuclei, however, have not been observed in this zone.

Other than the rami as just noted (fig. 27), an outgrowing axis cylinder with its end bulb, as described by Cajal ('07), has been observed only once by me. Figure 30—from a teased preparation of the sciatic nerve of No. 38—shows in *a*, a structure with an end bulb which stained intensely with phosphomolybdic acid hematoxylin and which is lodged in a band of poorly staining tissue rich in nuclei. Whether the band of tissue is a group of embryonic nerve fibers or non-medullated fibers in regeneration, I cannot say.

In the light of this group of evidence which bespeaks an outgrowth of the axis cylinder into the old nerve fiber sheath, certain observations of Cajal ('07), Marienescio ('06), Ranson ('12) and others gain an additional interest. These investigators found that, almost immediately after section, the axis cylinders of the central stump showed evidence of growth activity. "Marienescio, in one of his recently published papers has demonstrated that the lengthening of the regenerating fibers (i.e., axis cylinders) is demonstrable twenty-four hours after a nerve has been cut" (Halliburton). Ranson ('12) says:

On the first day after the lesion some of the axons grow out into the exudate and break up into many branches (fig. 16). Others on the first day, give off fine branches from their surface within the sheath in the immediate neighborhood of the lesion (fig. 17), some of which find their way into the exudate. Thus from the end of the first day on, fine nerve fibers, which are demonstrably branches of the medullated axons of the proximal stump, are present in the developing scar, and . . . running for the most part within the sheath of the old axon from which they arose, they arrange themselves into fascicles, etc.

Cajal's beautiful figures illustrate most clearly the axis cylinder growing down into the old sheath of that portion of the central stump which showed degeneration after section. Howell and Huber ('92) also observed a similar growth of the axis cylinder in the central stump. Branches of these axis cylinders are also to be seen growing up the medullated fiber in a central direction; others burst through the sheath into the interfibrillar tissue, and still others after invading the blood clot and inflammatory tissue between the sutured central and peripheral stumps

are seen to grow down into the old fiber sheaths of the peripheral stump. All this may take place before there is any marked increase in the number of nuclei of the neurilemma sheath and long before embryonic nerve fibers develop. It is further a common observation (as pointed out by Langley and Anderson ('04)) that, unless very special precautions are taken to prevent it, the peripheral stump is invaded by foreign fibers from neighboring nerves; and Forsmanns has shown that even macerated brain tissue exerts a chemotactic influence on the outgrowing axis cylinders.

Clearly then the importance per se of the embryonic nerve fiber in the regeneration of medullated nerves has been greatly overestimated.

To summarize briefly, there have been observed in the nerves of this series of fowls which have recovered from a pronounced paralysis of the legs brought on by a prolonged diet of white rice, the following: sections of nerves, taken from the sciatic and its peripheral branches at various times during recovery of the fowl, showed a greater percentage of fibers possessing axis cylinders in the sciatic than in its peripheral branches. In regeneration, a new axis cylinder was acquired by those nerve fibers in which a long series of observations prove that the axis cylinder and myelin sheath had undergone marked degeneration. The large globules and small droplets of degenerated myelin persisted several months after complete recovery of the fowl. Multiplication of the nuclei of the neurilemma sheath and the resulting embryonic nerve fiber were lacking or were of the greatest infrequency in regenerating as well as degenerating medullated fibers. A new axis cylinder and segments or fragments of the old and globules of degenerated myelin were found together in the same nerve fiber, whose neurilemma sheath showed no increase in its nuclei. A new axis cylinder, a branch of the same ending in a bulb, fragments of the old axis cylinder and globules of degenerated myelin (and with no multiplication of the nuclei of the neurilemma sheath) were all seen in the same portion of a regenerating fiber. Two axis cylinders in the same fiber and indications of an outgrowing axis cylinder were observed.

From these facts it is clear that neither the nuclei of the sheath of Schwann nor the embryonic nerve fiber could have taken any part in the formation of the new axis cylinder. Consequently auto-regeneration in so far as it signifies the formation of a new axis cylinder by the embryonic nerve fiber does not obtain with fowls in regeneration after paralysis from polished rice.

The same observations which show that the new axis cylinder, in these experiments, is not acquired through auto-regeneration, also demonstrate that it is attained by outgrowth. The presence of a new axis cylinder and segments of the old in the same *old* medullary sheath; the presence of two new axis cylinders in an *old* sheath, and the occurrence of a new axis cylinder with an outgrowing branch, and of an outgrowing axis cylinder with an end bulb, can only mean that, in the absence of auto-regeneration, the new axis cylinder has grown out from its central stump.⁶

Regeneration in the cord

Before considering the possibility of regeneration in the fibers of the cord, it is necessary to refer to the degenerative changes in the medullated fibers and nerve cells of the cord described by me in a recent study of "Polyneuritis Gallinarum" ('12). Here it was found that a very small per cent of the fibers of all columns of the cord presented as advanced myelin degeneration, as the fibers of the peripheral nerves. A still smaller per cent of the fibers also showed a disintegration or breaking up of the axis cylinder. The nerve cells of the ventral horn and the basal

⁶ The question naturally arises, at what point does this outgrowth of the axis cylinder begin? I have not been able to answer this satisfactorily. One would suppose that outgrowth would begin at the peripheral end of that segment of the axis cylinder (still connected with the nerve cell) which did not undergo degeneration; if such a segment exists. As stated below, I have not been able to determine if that portion of the axis cylinder running between the anterior horn cells and the periphery of the cord ever shows segmentation, such segmentation not having been observed. Both ventral and dorsal nerve roots, on the other hand, have been frequently observed in which segmentation and disintegration of the axis cylinder and clumping of the myelin were clearly visible.

portion of the dorsal horn experienced marked changes in their chromophile, Nissl, or tigroid substance, the globules or flocculi had given way to a uniform, finely granular mass collected at one side of the nerve cell, usually at the base of one of the processes of the cell. I was not able to determine whether that portion of the axone between the motor nerve cell and the periphery of the cord ever underwent segmentation.

A close examination of sections of the cords of Nos. 38, 57, 61 and 64 has revealed a persistence of globules of degenerated myelin in the medullated fibers of all columns of the cord for 59, 108, 275 and 379 days. These globules were frequently very large and occupied the entire diameter of the fiber. Furthermore, a careful search has failed to reveal any evidence of regeneration in the fibers of the cord. No such proof of regeneration as was found in the fibers of the sciatic and illustrated in figures 17 to 30 was found in the cord. Nothing suggestive of a new axis cylinder, an outgrowth or branching of the same was seen and there were not observed two axis cylinders in the same fiber.

On the other hand, fibers in degeneration in all columns of the cord were found in which no new axis cylinder nor fragments of the old were observed. Figure 31 is a cross-section of a degenerated fiber 49 days after complete recovery of fowl No. 38 (108 days in regeneration) was attained. A large globule of degenerated myelin completely fills up and distends the sheath and no indication of the axis cylinder is to be seen.

On comparing cross-sections of the lumbo-sacral cord of fowls taken at the time paralysis developed with sections from the same region of other fowls several months after complete recovery, the data included in table 1 were obtained.

From this table it will be seen that there are as many degenerated fibers with no axis cylinder in the cords of those fowls killed several months after recovery, as in the cords of fowls killed at the time paralysis developed.

In the absence of any positive evidence of regeneration, the persistence of degenerated fibers with no axis cylinder, in as great numbers as at the height of degeneration, strongly suggests the

conclusion that regeneration in the medullated fibers of the cord of the rice-fed fowls fails to take place.

Regeneration in the cord was included in this study because, after regeneration had been shown to take place in the peripheral nerves by an outgrowth of the axis cylinder into the old medullary sheath and in the absence of the embryonic nerve fiber, no reason was now apparent why the same thing could not occur in the fibers of the cord as well. According to the majority of investigators, regeneration in the fibers of the cord is, to say the most, doubtful. Schäfer ('08) declares "regeneration does not occur within the central nervous system, or at most in a very incomplete manner. This fact may be associated with the circumstance that the fibers within the spinal cord and brain have no nucleated sheath of Schwann, and the conducting path which the cells of this sheath form in the peripheral nerves for the outgrowing axis cylinders is therefore absent." According to Halliburton ('07), as noted above, the neurilemma is 'non-existent' in the medullated fibers of the central nervous system and "as is well known, regeneration does not occur" in these fibers. But

TABLE 1

Showing degeneration in the lumbo-sacral cord at time of paralysis and after recovery

FOWL NO.	NUMBER OF DEGENERATED FIBERS WITH NO AXIS CYLINDER IN THE CORD OF THE FOWL KILLED		REMARKS
	At time of paralysis	After complete recovery	
14	77		
1	65		
13	28		
38		{ 59 }	108 days in regeneration
		{ 67 }	49 days after recovery
57		62	379 days in regeneration
			300 days after recovery
64		57	275 days in regeneration
			256 days after recovery
61		117 ¹	59 days in regeneration; no recovery

¹ The cord of this fowl also contained a great many fibers which appeared considerably swollen and which are not included in this count.

as noted, if regeneration in the peripheral nerves was accomplished without the embryonic nerve fibers, then clearly in these fowls the absence from the cord of the neurilemma sheath and its derivative, the embryonic nerve fiber, would afford no explanation of a failure or regeneration in the fibers of the cord, and an outgrowth of the axis cylinder in the fibers of the cord might well be expected. Such, however, as the evidence shows, is probably never the case.

Although at the time of paralysis the marked changes described above were seen in the nerve cells of the lumbo-sacral cord, it is doubtful that this should be termed degeneration. The mitochondria seem to have undergone no alteration whatsoever. They were just as numerous as in the nerve cells of the cord of the normal fowl. In the cells of the cord of fowl 79, killed as soon as all signs of paralysis had disappeared (after 30 days on a special regenerative diet), the tigroid bodies again presented an appearance similar to the normal. This was also true for fowl No. 57 (10 months after complete recovery). No 'shadows' or other evidences of degenerated nerve cells were found in either case.

GENERAL SUMMARY

In the experiments described above degeneration of medullated nerve fibers was brought about in fowls by a prolonged feeding of polished rice, and regeneration was accomplished by a return to an adequate nutritive diet.

In such fowls the fibers are intact during degeneration and all traumatic and inflammatory effect produced by cutting the tissues and the nerve or of tying the latter are obviated; the process of degeneration can be stopped at almost any stage or greatly prolonged, and several stages of degeneration are to be observed in different fibers of the same nerve. In regeneration the possibility of an ingrowth of fibers from other nerves into the regenerating nerve under observation is eliminated and repair of the medullated nerves can be induced after any stage of degeneration.

Ten to 20 per cent of the medullated fibers of the nervus ischiadicus showed a complete fatty change of their medullary

sheaths into globules of degenerated myelin and a segmentation or granulation of their axis cylinders. No multiplication of the nuclei of the neurilemma sheath could be observed and consequently no embryonic nerve fibers or Band-fasern.

During recovery these degenerated fibers attained new axis cylinders and the medullary sheaths returned to normal. In other words, regeneration has been observed to follow degeneration in medullated nerve fibers without passing through the embryonic nerve fiber or Band-faser stage.

By prolonging the degenerative process there resulted a multiplication of the nuclei of the neurilemma sheath. This and other experiments described tend to show that the embryonic nerve fiber may be coincident with a late stage of degeneration in medullated nerve fibers. It may not represent an early stage of regeneration and its presence does not signify an attempt at regeneration on the part of the medullated nerve fiber.

In the absence of the embryonic nerve fiber, the degenerated myelin was absorbed with extreme slowness, persisting as droplets after 1 year and 14 days. On the other hand, where the embryonic nerve fiber was formed the degenerated myelin quickly disappeared from the fiber. The conclusion is reached that the proliferating nuclei of the neurilemma sheath participate in the resorption of the degenerated myelin.

In regeneration a new axis cylinder was attained by outgrowth and in the absence of the embryonic nerve fiber. The new axis cylinder grew down the old medullary sheath which latter still contained large globules of degenerated myelin and fragments of the old axis cylinder. The outgrowing axis cylinder was seen to branch, and in cross-sections of the nerves two new axis cylinders were observed within the same old medullary sheath. The embryonic nerve fiber could, of course, play no part in the formation of the new axis cylinder either by auto-regeneration or by outgrowth.

No indications of regeneration were observed in the fibers of the spinal cord.

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⁷ This list of references is meant to include only such of the available literature as has a direct bearing on the subjects discussed.

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PLATE 1^{*}

EXPLANATION OF FIGURES

1 Photomicrograph of a teased preparation of the nervus ischiadicus of a normal fowl. Marchi method. Zeiss 4 × 16 mm.

2 Photomicrograph of a teased preparation of the nervus ischiadicus of fowl No. 2, after 24 days on an exclusive diet of polished rice. Every fiber shows degeneration. Advanced degeneration is seen in 4 fibers. Marchi method. Zeiss 4 × 16 mm.

3 Fiber from the nervus ischiadicus of fowl No. 6—3 days in paralysis—showing marked degenerative changes, a swelling of the fiber at *a*, *b*, etc., and no nuclei of the neurilemma sheath. *m* is a hollow globule of degenerated myelin containing a segment of the axis cylinder. Marchi method. × 200.

4 Fiber from the nervus ischiadicus of fowl No. 1—showing complete alteration of the medullary sheath into globules of degenerated myelin. The laminated appearance of the larger globules is seen at *m*. *n* and *n'* are nuclei of the neurilemma sheath. Marchi method. × 312.

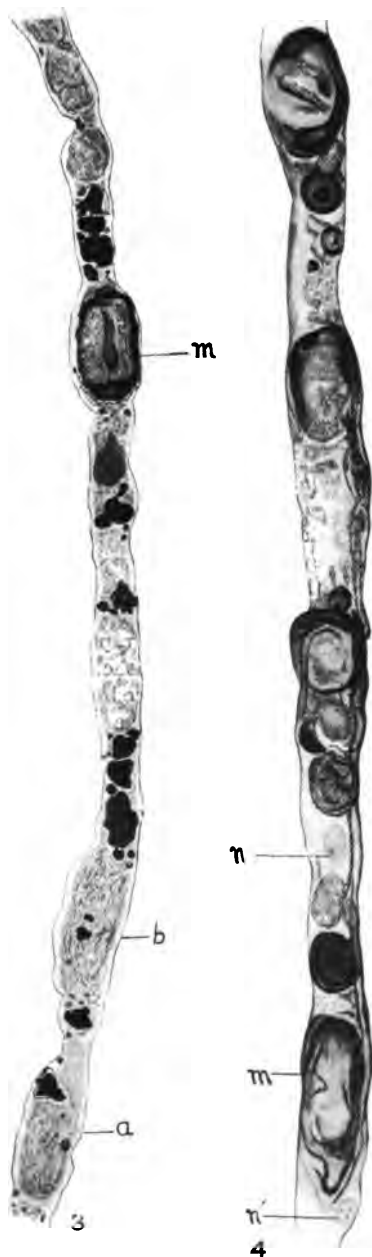
^{*} Camera lucida outlines were used in the preparation of all the drawings. The photomicrographs are by Martin and Cortes.



1



2



3

4

PLATE 2

EXPLANATION OF FIGURES

5 Fiber of the nervus ischiadicus of fowl No. 6. No multiplication of nuclei of the neurilemma sheath is seen. Marchi method. Zeiss 4×4 mm.

6 Photomicrograph of section of nervus ischiadicus of fowl No. 9, *G*—52 days on polished rice and calcium lactate. *a*, *a'* segments of discontinuous axis cylinders enclosed within enlarged portions (globules of degenerated myelin) of the fiber. Mallory's phosphomolybdic acid hematoxylin. Zeiss 4×2 mm. oil immersion.

7 Photomicrograph of teased preparation of nervus ischiadicus of fowl No. 6—24 days on polished rice. Several stages of degeneration are shown. Fiber, *a*, showing advanced degeneration lies side by side with one showing only slight degenerative change. Marchi method. Zeiss 4×4 mm.

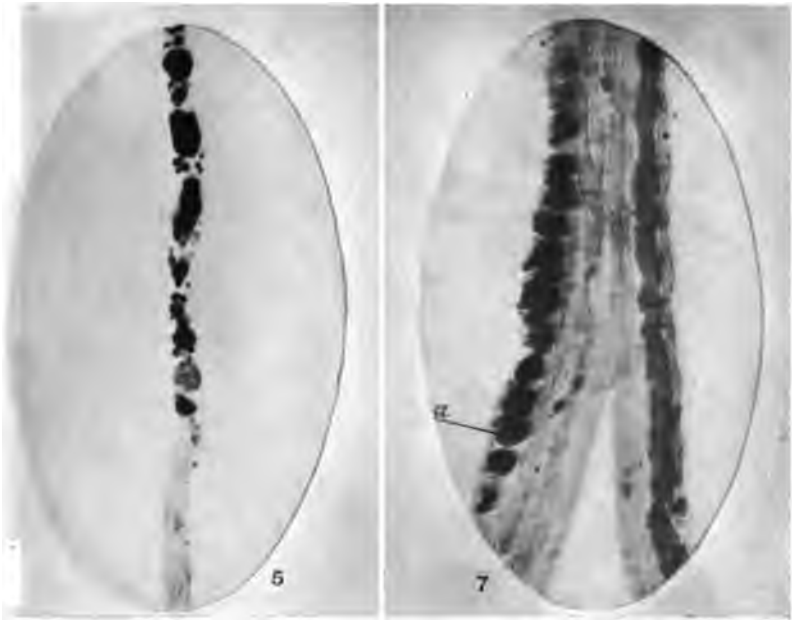


PLATE 3

EXPLANATION OF FIGURES

8 Photomicrograph of teased preparation of nervus ischiadicus of fowl No. 78—dry gangrene in left leg which had been bandaged for 24 hours 28 days before death. Slender embryonic nerve fibers with numerous spindle-shaped nuclei have entirely replaced the medullated fibers. Delafield's hematoxylin, Zeiss 4×2 mm. oil immersion.

9 Photomicrograph of embryonic nerve fibers in the nervus ischiadicus of fowl No. 17, *G*—60 days on polished rice and calcium lactate. Delafield's hematoxylin. Zeiss 4×2 mm. oil immersion.

10 Embryonic nerve fiber from the same nerve as figure 9. Droplets of degenerated myelin are seen at *m*. Delafield's hematoxylin. $\times 255$.

11 Embryonic nerve fiber from a teased preparation of the nervus ischiadicus of fowl No. 9, *G*—52 days on polished rice and calcium lactate; droplets of degenerated myelin are seen at *f*; *n*, nucleus. Delafield's hematoxylin. $\times 250$.

12 Fiber of the nervus ischiadicus of fowl No. 14, showing infiltration by numerous wandering cells. *S* is a nucleus of the neurilemma sheath. Delafield's hematoxylin. $\times 125$.

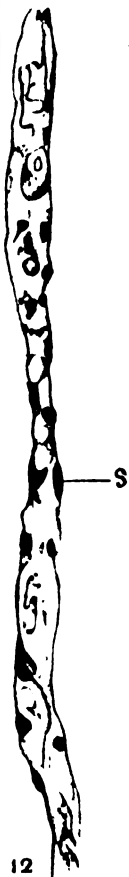
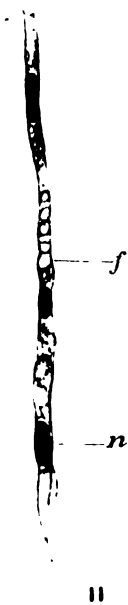
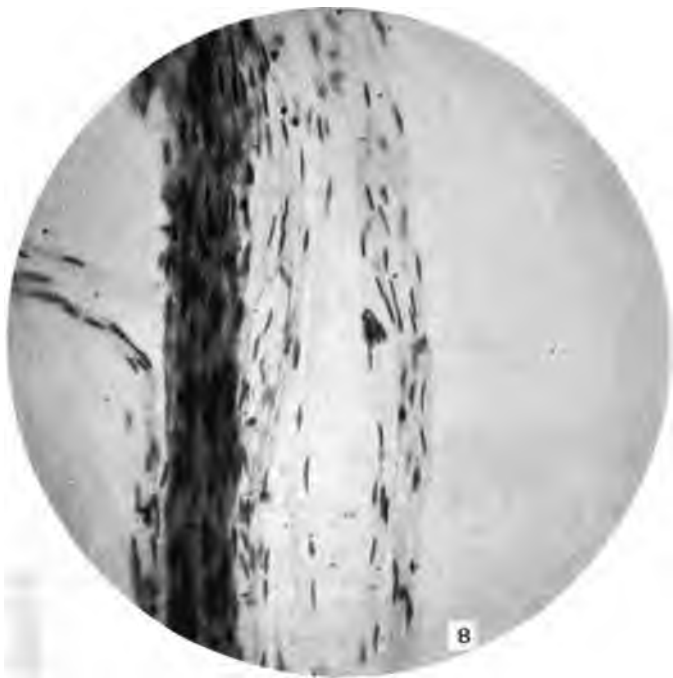


PLATE 4

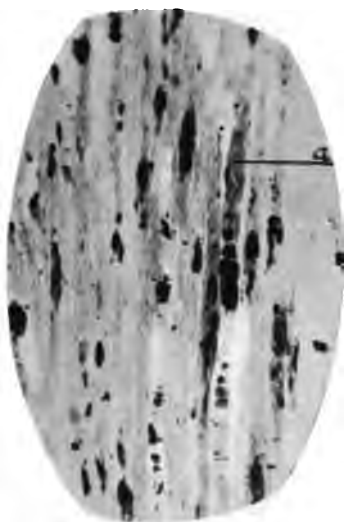
EXPLANATION OF FIGURES

13 Photomicrograph of a section of the nervus ischiadicus of fowl No. 38—108 days in regeneration and 49 days after recovery was apparently complete—droplets or globules of degenerated myelin are to be seen in every fiber. *a* resembles a fiber in advanced degeneration. To be studied in connection with figures 14, 15 and 16, showing the slowness of absorption of degenerated myelin. Marchi method. Zeiss 2×16 mm.

14 Teased preparation of nervus ischiadicus of fowl No. 54—171 days in regeneration. Marchi method. Zeiss 2×16 mm.

15 Teased preparation of a motor root of the nervus ischiadicus of fowl No 57—1 year and 14 days in regeneration—compare with figure 16. Marchi method. Zeiss 2×16 mm.

16 Teased preparation of sensory root of same nerve as figure 15. Marchi method. Zeiss 2×16 mm.



13



14



15



16

PLATE 5

EXPLANATION OF FIGURES

17 Fiber from the nervus ischiadicus of fowl No. 38—108 days in regeneration—*a*, new axis cylinder; *a'*, segment of old axis cylinder enclosed within a large globule of degenerated myelin, *m*; *n*, node of Ranvier. Mallory's phosphomolybdic acid hematoxylin. $\times 500$.

18, 19 and 20 Photomicrographs of sections of the nervus ischiadicus showing new axis cylinders, *a*, and fragments of the old, *a'*, in globules of degenerated myelin, *m*. Figures 18 and 19 from fowl No. 38. Figure 20 from fowl No. 54, 171 days in regeneration. Phosphomolybdic acid hematoxylin. Zeiss 4×2 mm. oil immersion.

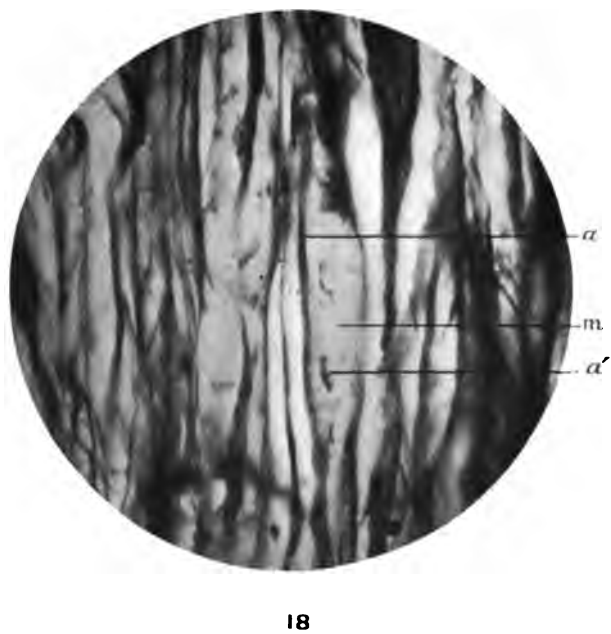
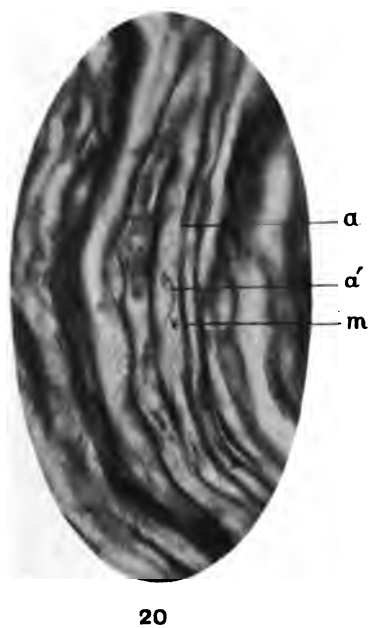
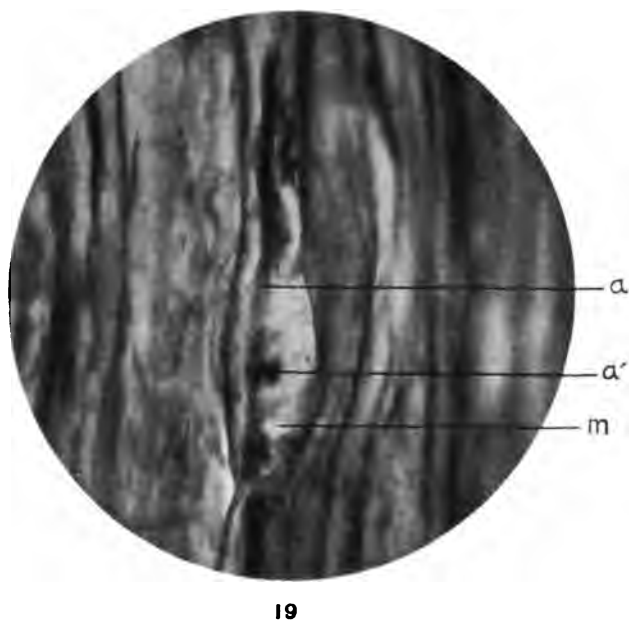
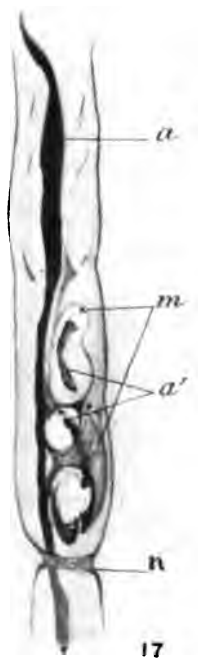


PLATE 6

EXPLANATION OF FIGURES

21 Cross-section of fiber of nervus ischiadicus of fowl No. 54. A new axis cylinder, *a*, lies adjacent to the old, *a'*, which still contains neurofibrillae. *m*, a globule of degenerated myelin. Phosphomolybdic acid hematoxylin. $\times 555$. (See also fig. 23.)

22 Cross-section of fiber of nervus ischiadicus of fowl No. 38. *a*, new axis cylinder with newly formed medullary sheath, *s*; *a'*, remnants of old axis cylinder; *n*, neurilemma sheath; *c*, connective tissue fibrils. Phosphomolybdic acid hematoxylin. $\times 1000$. (See also fig. 24.)

23, 24 and 26 Photomicrographs respectively of the same fibers shown in figures 21, 22 and 25. Zeiss 4×2 mm. oil immersion.

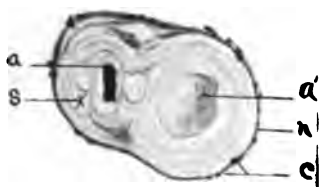
25 Cross-section of fiber of nervus ischiadicus of fowl No. 54. Two new axis cylinders, *a*, are seen within the old myelin sheath, *m*, and in the position previously occupied by the old axis cylinder. Each has acquired a secondary myelin sheath. Phosphomolybdic acid hematoxylin. $\times 1000$. (See also fig. 26.)



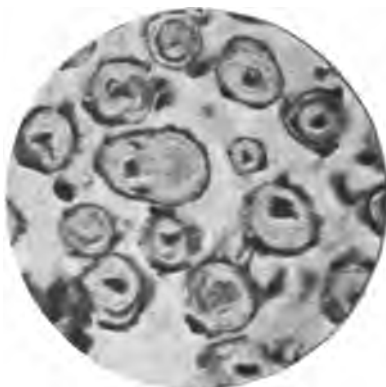
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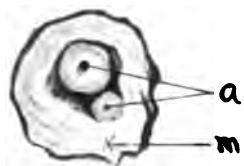
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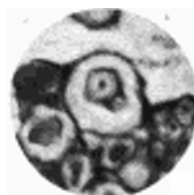
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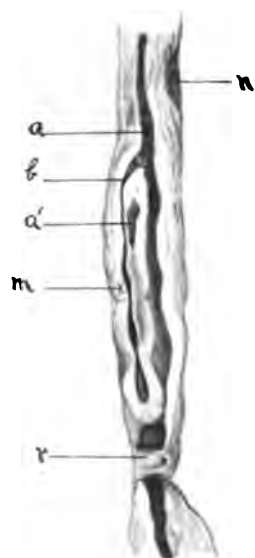
PLATE 7

EXPLANATION OF FIGURES

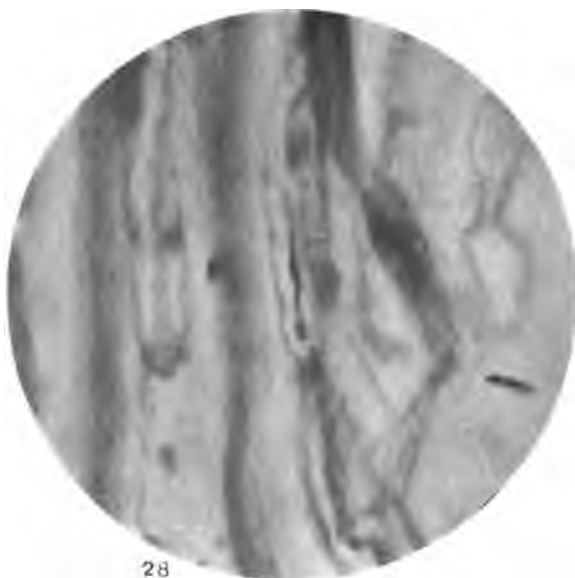
27 Fiber of the nervus ischiadicus of fowl No. 38. *a*, new axis cylinder with a branch, *b*, growing down into a long globule of degenerated myelin, *m*; *a'*, remnant of old axis cylinder; *n*, nucleus of neurilemma sheath; *r*, node of Ranvier. Phosphomolybdic acid hematoxylin. $\times 500$. (See also figs. 28 and 29.)

28 and 29 Photomicrographs at different focal levels of the same preparation shown in figure 27.

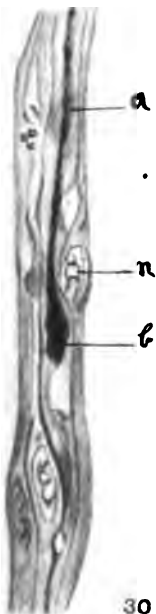
30 New axis cylinder, *a*, with end bulb, *b*, among a group of nucleated bands (embryonic nerve fibers or non-medullated fibers?). *n*, nuclei. Phosphomolybdic acid hematoxylin. $\times 555$.



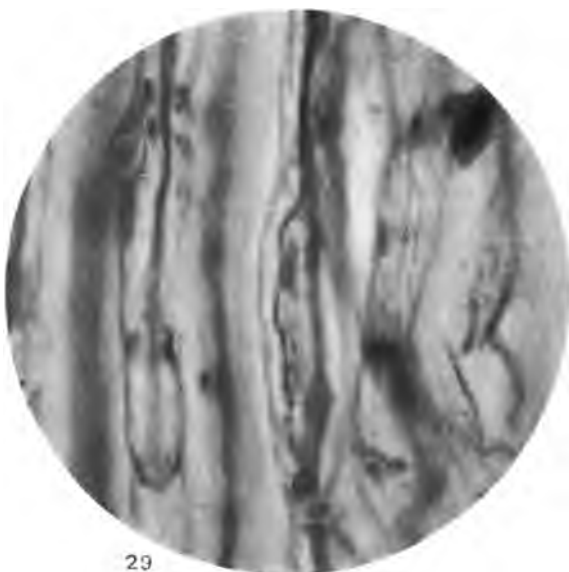
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PLATE 8

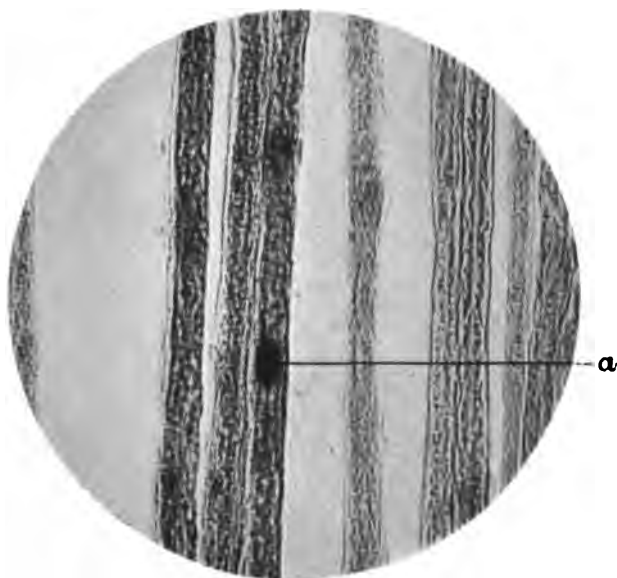
EXPLANATION OF FIGURES

31 Photomicrograph of a cross-section of fibers of the lumbo-sacral cord of fowl No. 38—108 days in regeneration—*a*, is a fiber greatly distended by a large globule of degenerated myelin. No indication of an axis cylinder is visible. Phosphomolybdic acid hematoxylin. Zeiss 4 × 2 mm. oil immersion.

32 Photomicrograph of teased preparation of nervus ischiadicus of fowl No. 24—7 days on polished rice. Two droplets of degenerating myelin are seen in fiber *a*. Marchi method. Zeiss 4 × 2 mm. oil immersion.



31



32

111

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THE DEVELOPMENT OF THE OLFACTORY NERVE AND ITS ASSOCIATED GANGLION IN LEPIDOSTEUS

CHARLES BROOKOVER

From the Anatomical Laboratory of the University of Arkansas

SEVENTEEN FIGURES

The writer (Brookover '08) published a preliminary note concerning the presence of a ganglion on the olfactory nerve of young specimens of *Lepidosteus* and in a later paper ('10) gave some additional facts concerning it. Considerable literature has grown up in relation to the nervus terminalis in recent years and there seems no doubt that this ganglion in the embryos is related genetically to the nervus terminalis. McKibben ('11) deals with the nervus terminalis, especially its central relations in the urodeles, and Herrick ('09) had previously noted its presence in *Anura*. McCotter ('13) has demonstrated it in adult dog and cat, and Johnston ('13) has described it in embryos of reptiles and mammals. The reader is referred to the bibliographies of these papers for the complete literature.

With the idea of shedding further light on the structure and possibly the function of this nerve, which is apparently universally present in the vertebrates, we have undertaken to follow the development of the nervus terminalis through to its adult structure in *Lepidosteus osseus*, an important member of the ancient order of *Holostei*.

Formerly we had nothing but a miscellaneous collection of embryos and young, but more recently we have had a graded series of embryos procured for us by stripping the eggs and fertilizing them simultaneously. It is this same series that Landacre ('12) used in describing the epibranchial placodes of *Lepidosteus*. The preservation was in fluid of Zenker at intervals of about six

hours until hatching, when the interval was gradually lengthened until eleven days after hatching. Staining in bulk over night with Delafield's hematoxylin diluted with nine times its bulk of water was used in preference to iron hematoxylin because it does not stain the yolk granules.

We shall commence our description at the age of 112 hours after fertilization, just after the head and tail have begun to rise from the yolk and the embryo measures 8 mm. in length. A section transverse to the neural tube, slanting slightly backward and upward reveals two thickenings in the ectoderm (fig. 1) which develop into the nasal capsules and the olfactory nerve. Hence the olfactory nerve may be said to be placodal in origin. The depth of the placode is but little more than twice the thickness of the adjacent ectoderm. The placode is rounded into contact with the neural tube along a part of its deepest border. This contact exists in four sections 6 micra in thickness. In other sections mesodermal elements intervene between the placode and the neural tube. Except for one small space in the next anterior section of this embryo, the membrane separating the placode from the neural tube is intact and everywhere as evident as in the drawing. Consequently it is doubtful whether the placode has any fibrous connection with the neural tube at this age. Mitoses are shown in the center of the placode and other karyokinetic figures are found in adjacent sections within the placode near its contact with the neural tube, indicating the region of cell proliferation at this stage of development.

Other stages earlier than 112 hours reveal the same essential structure except that the placode is not so thick. The whole embryo is more compact and the mesoderm is not so reticular as that dorsal of the placode (fig. 1) but more like that shown ventrally of the placode at 112 hours. The neural tube has the usual structure of early embryos with its central layer of germinal cells and its more external zone of nuclei of epithelial cells. Neither of these layers is thick and the external marginal velum is scarcely recognizable.

The next stage (fig. 2) is a little over 120 hours old. In the eight hours intervening since the last stage there has been a large

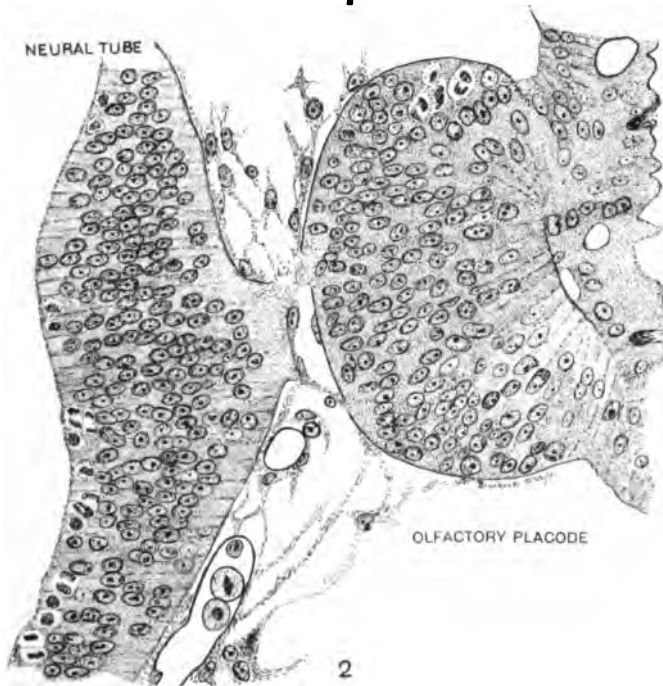


Fig. 1 Transverse section of an embryo 112 hours from fertilization with the olfactory placode in contact with the neural tube. $\times 400$.

Fig. 2 Embryo of 120 hours cut transversely showing two small rami of the olfactory nerve. $\times 400$.

increase in the number of cells in the neural tube and in the nasal placode but only a slight increase in the thickness of each. The embryo has increased scarcely at all in length, as indicated by a table of lengths made for the series of embryos, but it has expanded in the head region so that the mesenchyme forms an open network. This network fills a considerable space between the neural tube and the nasal placode. This space is narrowed somewhat where the neural tube rises up to meet the fibers of the olfactory capsule. The olfactory fibers are disposed in two rami which persist with considerable distinctness from this stage forward. Blood vessels can be recognized near the ventral root. A line of demarcation between the placode and the ectoderm appears and the latter has begun to vacuolize to form the external nasal opening. Mitoses are found in the deeper portions of the olfactory placode near the origin of the fila olfactoria, which occur in but two sections, but no karyokinetic figures are found in the neural tube beyond the germinal region close to the lumen of the canal.

Three intermediate stages have been studied but the same essential relations exist until we come to the age of 148 hours. In sections of this age a number of cells are to be seen on the ventral ramus of the olfactory nerve (fig. 3) at the point where it arises from the placode. If an occasional cell is found on the dorsal root, their numbers are never so great in any of the embryos of this age examined. However, it is only by reading from the older stages where there is no difficulty in recognition of these cells in a compact ganglion, back into the earlier stages that one would notice the greater number of cells on the ventral ramus. It has not been possible to discriminate between the mesodermal cells lying near the ventral ramus, and the ganglion cells at an early age, although a number of stains have been used upon the material at hand. Yet some of these cells lying among the fibers of the fila olfactoria are not mesodermal elements and are the earliest recognizable cells of the ganglion of the nervus terminalis.

Six hours later the cell aggregation at the surface of the ventral ramus (figs. 4 and 5) is not appreciably larger and the two adjacent sections drawn are the only ones showing any cells to be attributed to the ganglion of the nervus terminalis. The more

anterior of these two sections (fig. 4), which shows the ventral ramus cut obliquely in the distal portion of its extent, contains the greater number of cells. As in the previous stage, they are in close proximity to the surface of the placode. This section and the next anterior to it show a slight disturbance of the general epithelial arrangement of the cells within the placode adjacent to the

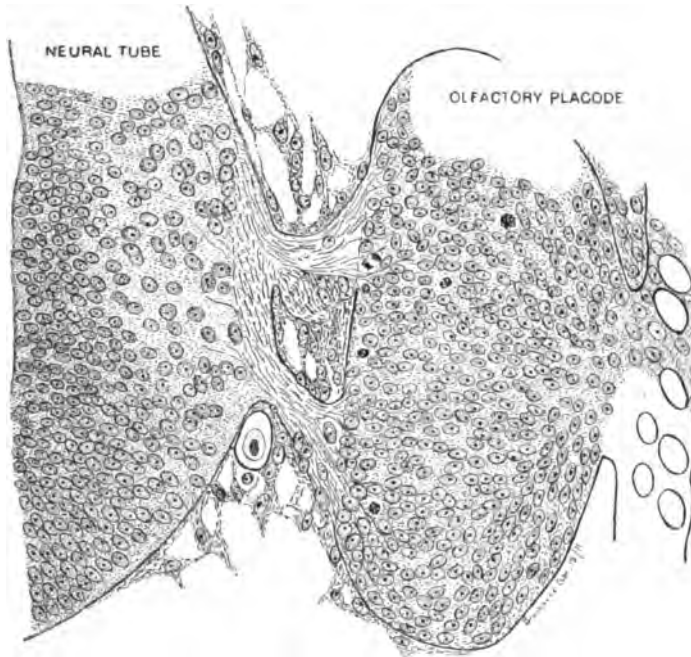
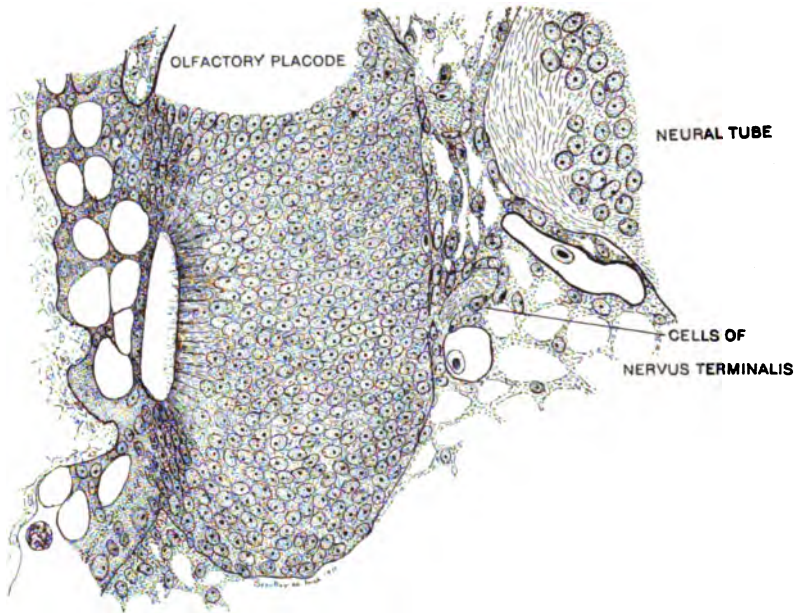


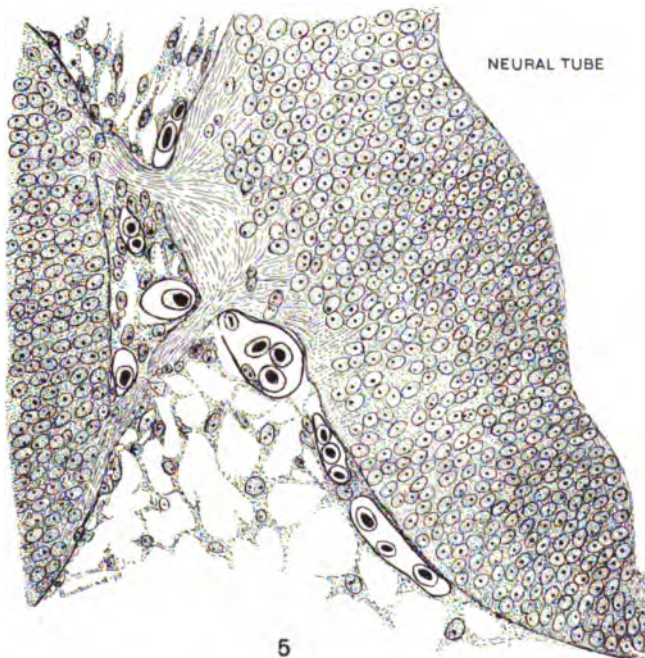
Fig. 3 Transverse section at 148 hours from fertilization, with cells in the ventral ramus of the olfactory nerve. $\times 400$.

origin of the ventral ramus. This might be interpreted as indicating a migration of the placodal cells into the ventral ramus. Blood vessels are in close proximity to the ganglion (fig. 4).

Six hours later, at the age of 160 hours from fertilization, a greater number of cells can be seen along the distal portion of the ventral ramus (figs. 6 and 7). Two facts are to be noted especially. The first is that some cells are slightly larger than those within the placode and are located among the distal olfactory fibers (fig. 6) as if they had migrated from the placode. The second is



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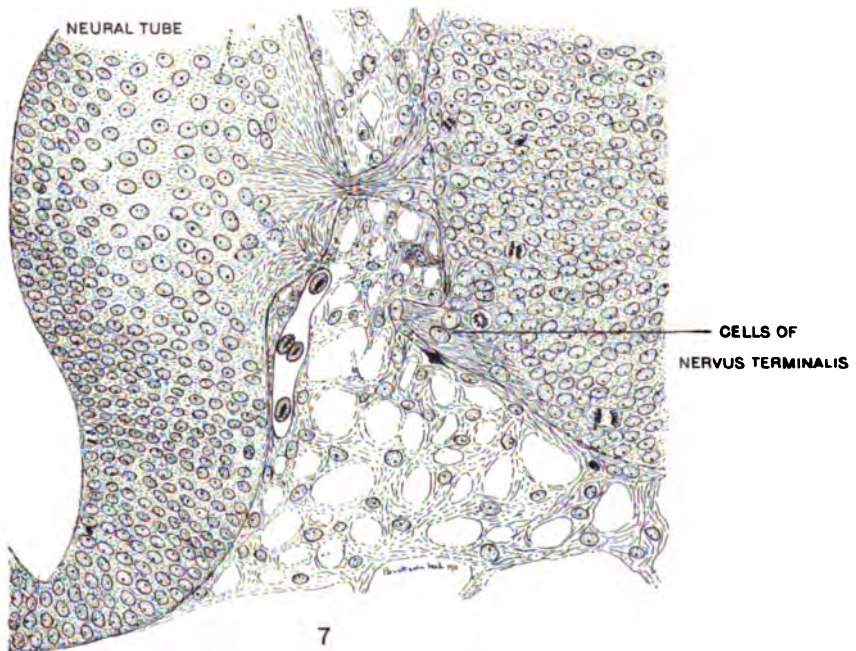
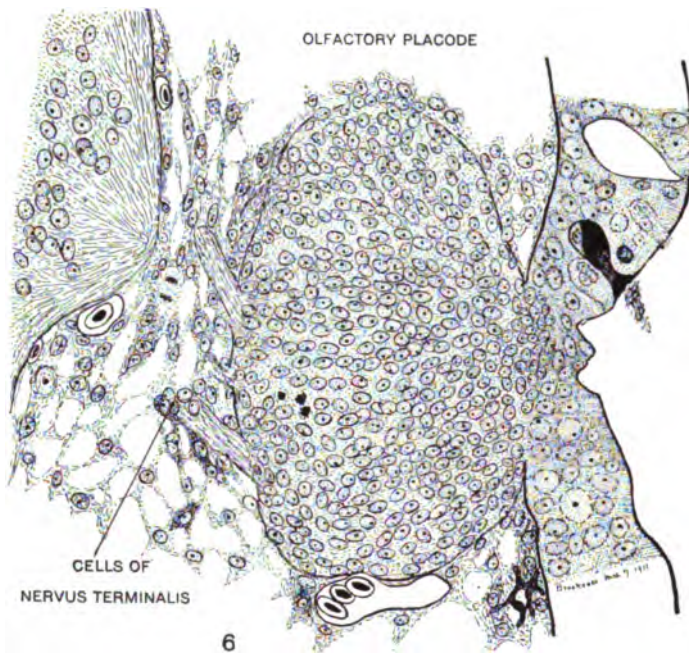
Fig. 4 Section through the brain and nasal capsule 154 hours after fertilization. Some cells are crowded about the ventral ramus of the olfactory nerve; blood vessels near. $\times 400$.

Fig. 5 Adjacent section of the same embryo as in fig. 4. $\times 400$.

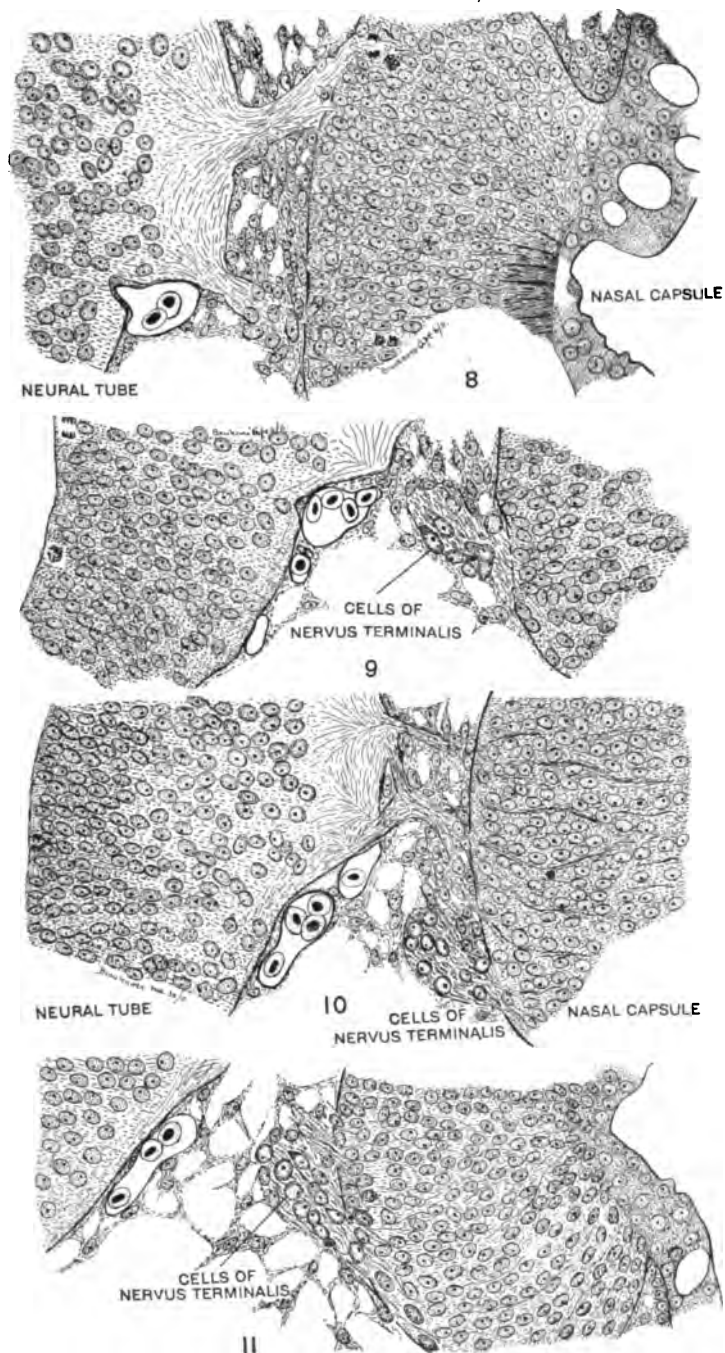
that mitoses are numerous in this region of the placode, contiguous to the origin of the ventral olfactory ramus. It is worth while to note that this is the next succeeding stage in the series to the 10 mm. embryo described by Landacre ('12, p. 6) as occupying an intermediate stage in the formation of the epibranchial placodes. We have then, a parallel history in the olfactory placode, for there is no difficulty from this stage on to the adult, in recognizing the cells of the ganglion of the *nervus terminalis*.

The next stage, no. 28 of the series, six hours later than the previously-described stage, and 166 hours after fertilization, is critical in reference to the formation of the ganglion. Four adjacent sections of the same embryo have been drawn and numbered consecutively from the most anterior to the most posterior in figures 8, 9, 10 and 11. Figure 8 shows a greater number of cells in the course of the ventral ramus of the olfactory nerve than in the dorsal ramus, just as noted in earlier stages. A blood vessel enters the brain opposite the ganglion and is a conspicuous landmark in later stages. The next posterior section (fig. 9) has a still larger number of ganglion cells in the ventral ramus, which is the only one drawn. The line of demarcation between the placode and the cells of the ganglion is not so marked as in the previous section. This line is less prominent in the next posterior sections (figs. 10, 11). In these posterior sections the number of ganglion cells is more numerous and they produce a slight swelling on the ventral ramus. They are slightly larger than a majority of the cells in the placode and rival those of the neural tube in size. But in the ventral part of the placode (fig. 11) are a few cells that are the same size as those in the olfactory nerve. They are on the border line or near it and appear to be migrating from the placode into the ventral olfactory ramus to become constituents of the ganglion of the *nervus terminalis*. This is the best and only evidence as to the origin of the ganglion of the *nervus terminalis* in *Lepidosteus*. At about this age the cells of the ganglion begin to acquire the characteristic vesicular nuclei of neuroblasts and chromatin granules are deposited in the cytoplasm.

A study of the following five stages of the series, covering a period of 48 hours, reveals the same essential relations to the



Figs. 6 and 7 Adjacent sections from an embryo aged 160 hours, with cells in the ventral ramus of the olfactory nerve and cell proliferation area adjacent within the nasal capsule. $\times 400$.



Figs. 8, 9, 10 and 11 Adjacent sections consecutive from before backward, through a 166-hour embryo, showing an increasing number of cells in the posterior part of the ventral olfactory ramus. These cells appear to be coming from the nasal placode and are recognized in all later stages as cells of the nervus terminalis. $\times 400$.

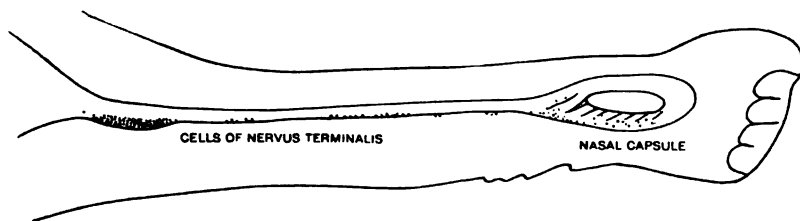
ventral olfactory ramus and the placode. The ganglion has increased in size so that it is found in as many as eight sections 6 micra thick, but does not exceed perhaps forty or fifty cells in number. They are readily distinguishable in most preparations by their deep staining qualities, as compared with surrounding elements like the mesenchyme from which in some cases the ganglion appears to be separated by a membrane. In most cases the ganglion is compacted into a spherical mass, but in other cases it is considerably elongated in the same direction with the length of the olfactory ramus, and in one case showed the appearance of two ganglia. As no models of this age were made and the sections are oblique to the nerve, the appearance of two ganglia may be an illusion. The ganglion lies nearer the olfactory placode in the earlier of these stages but comes to occupy a position about midway between the placode and the neural tube in the later stages which are nine days old. This is some days after hatching, when the nose has begun to elongate and the placode—or more properly, the olfactory capsule—lies anterior to the forward end of the neural tube. The opening of the nasal capsule remains single for a considerable time after this stage, which has a total body length of 13.5 mm.

We have sectioned the two or three specimens available from this series of embryos at the age of 272 hours from fertilization, when they are nearly eleven days old, 48 hours older than the last stage described. The length has almost doubled in the two days and totals 24 mm., with the sucking disk decadent. No doubt part of this rapid growth in length is due to the growth of the snout, carrying the nasal capsules far in advance of the brain (fig. 12). We have studied a transverse series of this age and it reveals the same distribution of cells of the nervus terminalis as is shown in figure 12, which is a flat reconstruction from a number of sagittal sections. The main ganglionic mass of cells is located in a swelling near the brain, on the ventro-median side of the olfactory nerve. The black dots peripherally along the nerve indicate the position and approximate number of cells outside the ganglion that are to be attributed to the nervus terminalis. As material was not at hand for the application of neurological

methods we have had to rely for recognition on the larger size and the deeper staining of the cytoplasm in hematoxylin preparations. In sagittal sections the neurological characters of vesicular nuclei and of cytoplasmic granules appear fairly well-marked. The granules (Nissl bodies) are more apparent than in transverse sections, since they are more numerous in the ends of these cells, which are elongated in the direction of the axis of the olfactory nerve. A slight increase in the number of cells at the posterior part of the nasal capsule might be designated a peripheral ganglion (fig. 12).

We have been fortunate enough to catch a few young *Lepidosteus* of varying sizes larger than the embryos just described, during our stay at the Ohio State University Lake Laboratory at Cedar Point on Lake Erie the past summers. The young are solitary in their habits and it has not been possible to secure them in large numbers for exhaustive neurological technique as with *Amia* ('10) and *Ameiurus* (Brookover and Jackson '11). We have studied transverse sections of a specimen 43 mm. long. A flat reconstruction of the proximal portion of the olfactory nerves as seen from the ventral side shows the position of the ganglia and the approximate number of cells is indicated by the dots (fig. 13.) There is a slight asymmetry in the form and position of the two ganglia, as might be expected where organs are slender, and this lack of symmetry was noted in specimens older and younger. The ganglia are rostral of the brain a distance of between one and two millimeters, the total length of the olfactory nerve being about 8 mm. at this age (fig. 13).

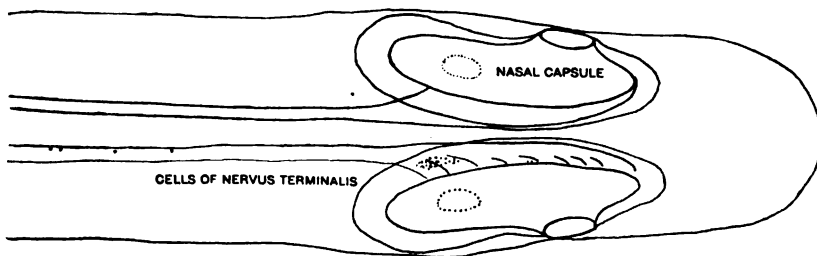
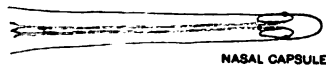
A flat reconstruction of the rostral end of the olfactory nerves and the nasal capsules of this 43 mm. specimen (fig. 14) as seen from below, shows a few cells (indicated by dots) that would seem to belong to the *peripheral* ganglion of the *nervus terminalis* mentioned in previously-described stages. These cells are located, for the most part, near the posterior end of the nasal capsule where the olfactory fibers begin to spread for their final distribution in the capsule. One side of the specimen was defective from interference of ossification in sectioning and no cells are indicated in the drawing (fig. 14); probably, moreover, not all the cells



12



13



14

Fig. 12 Flat reconstruction from sagittal sections of a 24 mm.-long *Lepidosteus*, 11 days from fertilization, seen from the medial side. Dots show the distribution and approximate number of cells of nervus terminalis. $\times 40$.

Fig. 13 Flat reconstruction from transverse sections of a 43 mm.-long *Lepidosteus*, seen from ventral side, showing the asymmetric central ganglia of the nervus terminalis on the ventral side of the olfactory nerve. Diagram adjacent to show on smaller scale the positions of central and peripheral ganglia (see fig. 14) with reference to the brain and nasal capsules at this age. $\times 27$.

Fig. 14 Flat reconstruction of the nasal capsules of the fish used for figure 13, seen from the ventral side. Dots show the relative number and position of the cells of the peripheral ganglion on one side, the other being defective in the preparation. $\times 27$.

present are represented on the other side, for two reasons. As already stated, cross-sections are not favorable for the differentiation of these cells from others, and, in the second place, it is not easy to find them all, scattered as they are shown to be in the previous stage drawn (fig. 12) through the whole ventral portion of the nasal capsule. A very few cells were found proximally along the ventro-median border of the olfactory nerve not far from the nasal capsule (fig. 14.)

In a young *Lepidosteus* 85 mm. in total length cut sagittally, the central ganglion of the *nervus terminalis* was found 3 mm. rostral of the olfactory bulbs, a distance of 15 mm. posterior from the nasal capsules at a point slightly anterior to the masticating muscles of the jaw. The olfactory nerve on this (the right?) side of this fish has three peripheral ganglia with a total number of cells about equal to those in the central ganglion just mentioned. One is located 2 mm. posterior to the olfactory capsule, and the other two near each other, 5 mm. caudal of the olfactory capsule. No ganglion cells were recognized within the confines of the nasal capsule in this instance. It will be noted that there is a space of 10 mm. at about the middle of the length of the olfactory nerve, now some 18 mm. in total length, in which no ganglion cells were found.

The central ganglion on the other olfactory nerve of this 85 mm. fish was recognized in the same position as above indicated, but peripherally there was but a single ganglion found. It is located 1 mm. caudal to the olfactory capsule. Its size was estimated to be equal to the three combined peripheral ganglia of the opposite nerve. The olfactory capsule was so disturbed in the making of the sections that it was not possible to determine whether any ganglion cells were included within it.

In a transverse series of a fish about 90 mm. in length the central ganglia were recognized at a distance of 2 mm. anterior to the olfactory bulbs. In a specimen about 125 mm. in length the central ganglion was found on one side, situated 3 mm. rostral of the olfactory bulbs (fig. 15). This ganglion was estimated to have about 100 cells, occupying a ventral position on the olfactory nerve (fig. 16) between a large blood vessel laterally and a mass of carti-

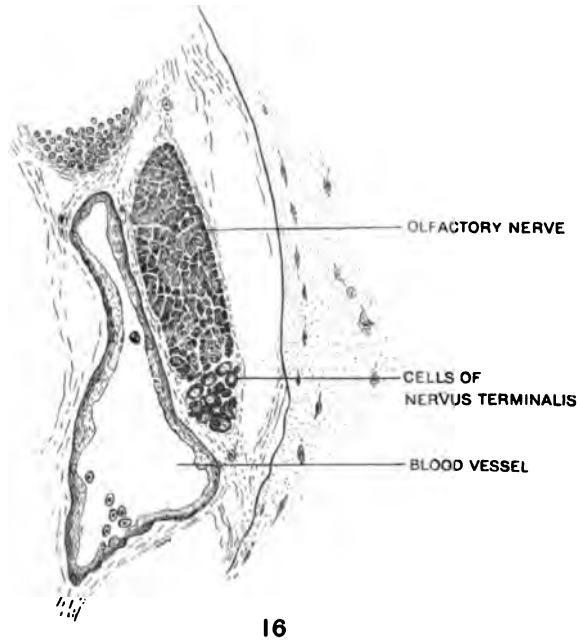
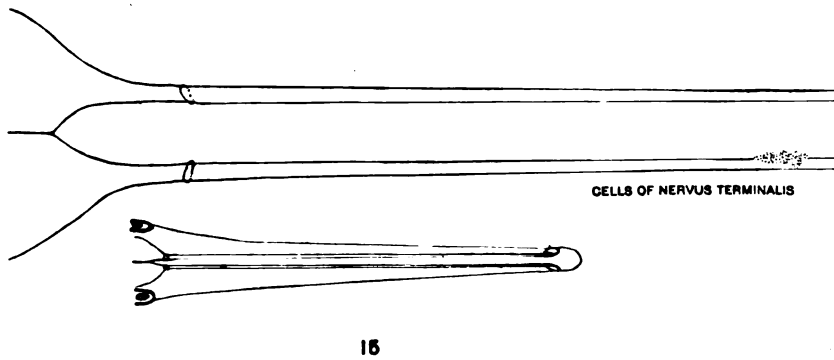


Fig. 15 Flat reconstruction from transverse sections of a 125 mm.-long *Lepidosteus*, as seen from below showing the position of the central ganglion of the nervus terminalis. Adjacent diagram to show on smaller scale the relative position of this ganglion to eyes, brain and nasal capsules. $\times 27$.

Fig. 16 Drawn from one of the transverse sections through the central ganglion shown in figure 15. The position of the ganglion is the same as in the adult and the relation of the blood vessels and the ramus ophthalmicus (not labeled in the upper portion of the drawing) is essentially the same as in the adult. $\times 200$.

lage medianly. It appears to be almost distinct from the olfactory nerve by reason of a weak investing membrane. Dorso-lateral to the olfactory nerve is a medullated nerve which is probably the ophthalmic branch of the V and VII cranial nerves. It may be significant that the central ganglion of the nervus terminalis is located not far from the place where the ophthalmic ramus joins the olfactory nerve on its way forward. This would provide connection posteriorly with the sympathetic system, if such a connection exists. The ophthalmic ramus runs parallel with the olfactory in close juxtaposition as far forward as the olfactory capsules. This transverse series is so defective in the region near the olfactory capsules that no peripheral ganglia were found on either side.

When the adult fishes were examined, the central and peripheral ganglia of the nervus terminalis were found to occupy the same relative positions as in the young fishes of 90 and 125 mm. length, just described. The central ganglion is generally found about 25 mm. rostral of the brain, immediately anterior to the masticating muscles. It has a ventral position on the olfactory nerve near the blood vessels. Figure 16 would serve to represent a cross-section of the adult, if the ophthalmic ramus were moved ventrally and laterally and the olfactory nerve were made larger. The ganglion contains not over two hundred cells. Search was made for any connection of the nervus terminalis with other nerves of the head. The ophthalmic branch already mentioned is compacted within the same bone-covered channel so that to the naked eye the olfactory nerve and the ophthalmic seem to be in contact. In sections of the adult the distance of the ophthalmic ramus from the ganglion of the nervus terminalis is 1 mm. In vom Rath preparations of the adult a slender nerve, of medullated fibers for the most part, was found in contact with the olfactory nerve at a point where there were five or six large ganglion cells. Whether these were outlying cells of the central ganglion of the nervus terminalis could not be established with certainty.

It has been impracticable to search the whole length of the olfactory nerve of the adult fish for ganglion cells, but we have examined the nasal capsules. In some cases no peripheral gan-

glia were found. Perhaps in these instances the ganglionic mass was not included on account of its posterior position along the olfactory nerve, as in the previously-described fish measuring 85 mm. in length. In figure 17 is shown, by a flat reconstruction from sagittal sections stained with toluidin blue, the distribution of what were interpreted as peripheral ganglia of the *nervus terminalis*. A similar distribution was found in a series treated by the vom Rath method. In these specimens one or two larger clumps of ganglion cells are embedded in the main olfactory nerve or between its main rami, and other smaller ganglia are situated

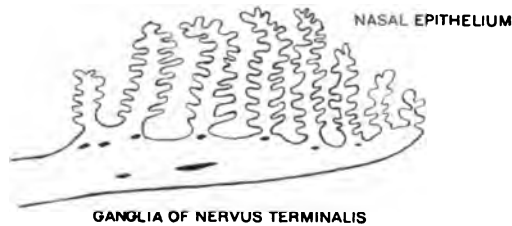


Fig. 17 Flat reconstruction from sagittal sections of the nasal capsule and olfactory nerve of adult fish to show the relative position of the peripheral ganglia of the *nervus terminalis* to the olfactory nerve and nasal mucosa. $\times 10$.

near the base of the main folds of the olfactory mucosa, along the branches of the olfactory nerve (fig. 17). These peripheral ganglia did not show well-marked Nissl granules, but this was attributed to the fact that the fishes available were kept empounded for some time and were not in the best of physical condition. However, there seems to be little doubt that these are ganglion cells, when we consider the embryological history given above and the similar position of ganglion cells in other fishes.

In the way of summary and conclusion, we may say in brief that in *Lepidosteus* the *nervus terminalis* ganglion cells seem to arise from the olfactory placode in a way similar to that described by us for *Amia* and *Ameiurus* in previous papers. The ganglion appears late in embryonic history, in close relation to the vascular tissues, and still later, in *Lepidosteus* becomes divided into a

fairly compact central ganglion and one or more peripheral ganglia persisting through all stages to the adult.

Belogolowy ('12), who has followed the development of the *nervus terminalis* in selachians, finds a ganglion to originate from the olfactory placode (p. 8) in accord with my description above, but would interpret this ganglion as representing in part a primitive olfactory ganglion (p. 11). As far as the majority of the embryological evidence goes, therefore, we may say that the *nervus terminalis* is a component of the olfactory (or primitive olfactory) nerve, and this might very well be the case, although it does not have the same central connection with the brain, as Johnston ('13, p. 108) has very well said. As to the function of the *nervus terminalis*, the present investigation has no new evidence. As we ('10) previously stated for *Amia*, the circumstantial evidence leads one to ascribe a vasomotor function to it in part. The disposition of the cells in *Lepidosteus* in more compact central and diffuse peripheral ganglia allows of its falling quite naturally into the morphological relations of the typical autonomic system. It may be noted that Huber and Guild ('13, p. 272) are somewhat inclined to assigning it to the sympathetic system.

I wish to acknowledge my indebtedness to the Ohio Academy of Science for a grant from the MacMillin Fund to defray the expense of collecting material, and to my wife for helping with the drawings.

Little Rock, Arkansas, January 14, 1914

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THE NERVUS TERMINALIS IN ADULT MAN

CHARLES BROOKOVER

*A preliminary account from the Anatomical Department of the Medical Department
of the University of Arkansas*

THREE FIGURES

Knowing that the nervus terminalis had been found in adult mammals by McCotter ('13) and others, and in human embryos by Johnston ('13) we decided to look for the nerve in human fetuses and in adults. Sections through the head of a fetus measuring 38 mm. head-rump length revealed essentially the same relations of the cells of the nervus terminalis as that described by Johnston for a slightly smaller embryo. Sections of the region containing the fila olfactoria and the nasal mucous membrane of fetuses of 90 mm. and 130 mm. head-rump length showed the nerve cells of the nervus terminalis to have increased in number intra-cranially as well as peripherally. Later we discovered these large nerve cells in sections of the adult fila olfactoria and the dura mater in the region of the cribriform plate of the ethmoid bone.

Most of the cells of the nerve in adult man found up to the present time are located median to the olfactory bulbs on the surface of the dura mater or embedded within the dura as far ventrally and peripherally as the cribriform plate. The number is estimated to be between one and two hundred. As in many other forms of vertebrates, these cells are located along a bundle of fibers appearing similar to the fila olfactoria with their characteristic sheath cells. This bundle keeps a median position to the fila olfactoria, but is not distinct enough peripherally to be followed in any of the series of sections so far made to permit of its being traced into the nasal mucous membrane as a separate and distinct bundle.

A few cells have been found in the sections so far made of the olfactory region of the nasal mucous membrane with its subjacent structures as far centrally as the cribriform plate. The number discovered is not as great as it would seem from sections of the fetuses above mentioned should be the case, since the number of cells attributable to the nervus terminalis peripherally in these fetuses appear to outnumber those within the cranial cavity. From the distribution of the cells of the larger of these fetuses it would appear that the cells are much scattered and that they are to be found in small numbers along many of the fila olfactoria, those distributed to the lateral nasal mucosa as well as those to the region of the nasal septum. From a study of a fetus which measures over five inches head-rump length, it is evident that the cells recognized as nervus terminalis cells extend beyond the olfactory into the respiratory region along the septum, and curiously enough, most of the cells noticed were found along a ramus traced anteriorly and ventrally to what was believed to be the rudimentary organon vomeronasale. That these cells should be found in the respiratory region and more numerous in the strand of fibers to Jacobson's organ corresponds with the condition found by Huber and Guild ('13) in the rabbit.

After some futile search for the central course of the nervus terminalis in close juxtaposition to the olfactory tracts we found its intra-cranial course posterior to the olfactory bulbs to lie over the middle of the gyrus rectus as shown in figure 1. A rectangular piece of the pia mater stripped from this region of the gyrus rectus has never failed to reveal the fibers of the nerve in all the well preserved brains examined. In some brains where the bundle is well compacted into a single strand it is possible to dissect the nerve out in situ with the aid of a small magnifier. As a general thing we have found the fibers to be broken up into two or three strands and especially is this the case at its anterior extent near the olfactory bulbs and posteriorly near the medial olfactory striae. The fibers of the nerve seem to have passed over from the dura to the outer surface of the pia mater in the region of the olfactory bulbs and to continue posteriorly external to the pia, but beneath the larger blood vessels of the arach-

noid. The nerve bundles take a fairly direct path here and do not seem to be influenced by the course of the vessels.

When *toto* mounts and sections of the meninges of the region just mentioned above are studied, it is clear we have a bundle of nerve fibers belonging to the *nervus terminalis* and not a strand of connective tissue. In the first place, the bundle occupies a definite position with characteristic method of occasional splitting and reunion of its strands. There is to be found a large number of sheath cells identical with those found in the *fila olfactoria* and about the cells of the *nervus terminalis* peripher-

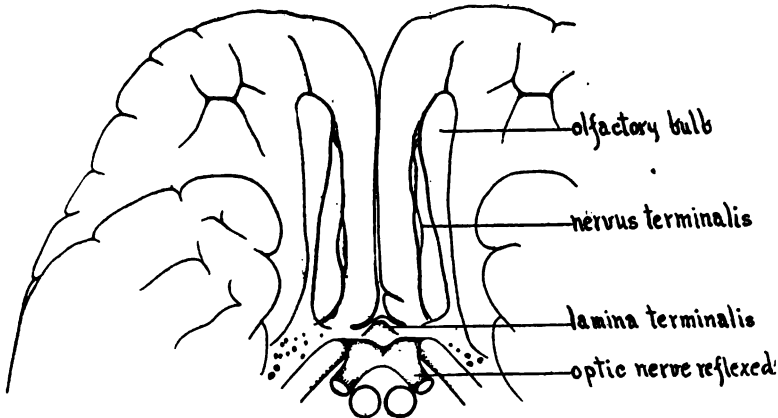
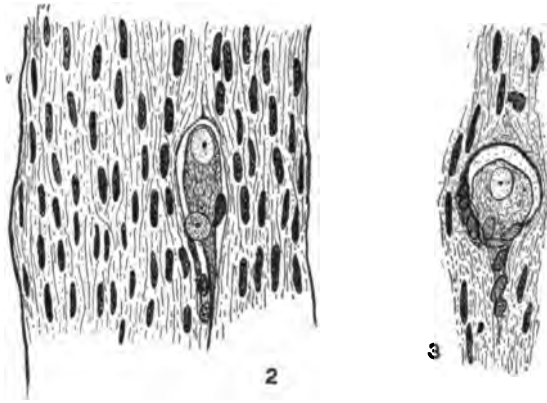


Fig. 1 Outline of the ventral surface of adult human brain, two-fifths natural size, from a rather small brain, to show the position of the central portion of the *nervus terminalis* and its relation to the olfactory tracts and the gyrus rectus.

ally. These sheath cells are deeper staining than the adjacent meningeal connective tissue fibers, and the fibrillar appearance of the nerve fibers among them is different from the larger and clearer connective tissue fibers. Perhaps the most conclusive proof that we are here dealing with a nerve and that it is the *nervus terminalis*, is the presence of ganglion cells so characteristic of the *nervus terminalis* in most, if not all, the vertebrates hitherto described. Two of the cells located posterior to the olfactory bulbs have been sketched in figures 2 and 3. In this region of the nerve they have not been found in greater numbers

than two or three in any given group. Perhaps the total number posterior to the olfactory bulb does not exceed thirty in adult man. A few were found well within the confines of the nerve, the whole width of which is shown in figure 2. More frequently they are near the boundary of the nerve, as has frequently been remarked for them in various vertebrates. In all cases noted there is a capsule about the individual nerve cell and in some cases the cells somewhat similar to the sheath cells elsewhere found in the nerve were found participating in the make-up of the cell capsule.



Figs. 2 and 3 Drawings from camera lucida outlines to give the relations of the sheath cells and ganglion cells to the nervus terminalis at about the middle portion of its extent from the olfactory bulbs posteriorly, lying immediately external to the pia mater. $\times 450$.

We are scarcely able to determine the posterior limit and brain connection of the nerve by gross dissection in situ with the aid of the hand lenses at our disposal, but my assistant, Mr. Dickinson, who has so kindly made some dissections of the nerve, has been able to show that in the cases examined the nerve breaks up into two strands in the region over the medial olfactory striae and some of the fibers seem to enter the brain substance in this region not far from the anterior median margin of the trigonum olfactorium. Sections are now being made to determine, if possible, whether all or a part of the fibers enter and in what rela-

tion to other structures of the brain. This region seems to correspond very well to the entrance of the nerve in other mammals, viz., not very far lateral to the lamina terminalis.

It will be interesting to note what relation, if any, the fibers of the nervus terminalis bear to any other fibers of nerves posterior that may enter the cranial cavity with the internal carotid, for I believe it is conceded that nerves accompany the vessels to the meninges. The internal carotid in man divides into anterior and middle cerebral arteries immediately over the most posterior point to which we have traced the nervus terminalis. The naso-ciliary branch of the ophthalmic ramus of the trigeminal nerve enters the cranial cavity lateral to the olfactory bulbs to pass out anteriorly into the nasal mucosa. Our sections of this nerve in its intra-cranial course in the adult and in its proximal distribution in the nasal mucosa did not reveal any cells that were like those of the nervus terminalis or that could be called ganglion cells. We are not clear from our sections that any branch is sent off from the intra-cranial course of the naso-ciliary.

Little Rock, Arkansas,
March 3, 1914.

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THE PYRAMID TRACT IN THE RED SQUIRREL
(SCIURUS HUDSONIUS LOQUAX) AND CHIP-
MUNK (TAMIAS STRIATUS LYSTERI)

SUTHERLAND SIMPSON

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THIRTY-SEVEN FIGURES

INTRODUCTION

The nerve fibers which form the pyramid tract take origin from the large pyramidal cells of Betz ('09) in the cerebral motor cortex. Entering the corona radiata they converge and pass caudalward through the internal capsule, crusta, pontine bundles and anterior pyramid until the lower part of the medulla oblongata is reached. The tract, in its passage downward, gives off fibers or collaterals to the optic thalamus, substantia nigra, nuclei pontis of the same side, and to the nuclei of the cranial motor nerves mainly of the opposite side. The positions occupied by the pyramid tract at these different levels are similar in all the mammalian orders that have been investigated, with slight variations, until the lower part of the bulb is reached, but beyond this, where it passes into the spinal cord, great differences are found to exist.

In man and the anthropoid apes, at the decussation of the pyramids the fibers divide into three bundles; most of them cross the middle line and enter the dorsal portion of the lateral column of the opposite side, forming the crossed pyramid tract of the cord. A second group of fibers, much less numerous than the first, turn away from the median raphé on the same side, without crossing, and take up a position in the lateral column corresponding to that of the crossed pyramid tract of the opposite side; this is termed the direct lateral pyramid tract. A third group continue downward from the pyramid into the cord,

running along the margin of the ventral longitudinal fissure, forming the direct ventral pyramid tract otherwise known as the bundle of Türck. This last can be followed to about the middle of the thoracic region. It is said to be wanting in all mammals below the anthropoid apes; such, however, is not the case, since it is present as a well-marked tract in the porcupine (*Erethizon dorsatus*, Linn.) and to some extent in the raccoon (*Procyon lotor*, Linn.), as shown by Simpson ('12).

In the monkey, cat, dog and rabbit the same subdivision takes place at the decussation as in man, except that the direct ventral pyramid tract is absent.

The few species of ungulates that have been examined show that the cortico-spinal fibers of the tract are practically absent and those that do reach the cord cannot be followed beyond the upper cervical segments. Ziehen ('00), in the sheep, says that they decussate, some passing into the dorsal and some into the lateral columns. Dexler and Margulies ('06), in the sheep and goat found that the decussation is not complete; most of the fibers cross and pass into the lateral column of the opposite side; some, however, remain in the ventral column of the same side. King ('11), in the sheep, found also an incomplete decussation; the crossed fibers passed into the lateral column of the opposite side, the direct fibers into the corresponding column of the same side. None could be traced beyond the first cervical segment. Bischoff ('00), in the pig and deer, was unable to say whether the crossed fibers pass into the lateral or the dorsal column.

All are agreed, at any rate, with regard to the ungulates, that the fibers entering the cord are extremely scanty and terminate early.

The insectivora and chiroptera—hedgehog, mole, bat—show similarly an almost complete absence of cortico-spinal fibers, and the very few that do enter the cord end in the first cervical segment. Whether the decussation is complete, partial or entirely absent is still an open question: Bischoff ('00), Ziehen ('99), Kotzenberg ('99), Obersteiner ('03), Edinger ('11), Van der Vloet ('06), Dräseke ('03), Merzbacher and Spielmeyer ('03), and Hatschek ('03).

In the monotremes and marsupials the fibers are said to decussate into the dorsal columns. Ziehen ('99) found it to be so in the ring-tailed phalanger (*Pseudochirus peregrinus*). In another species, the koala (*Phascolarctus cinereus*), he was unable to say whether the fibers, after crossing, pass into the dorsal or lateral column. Edinger ('11) describes complete decussation into the dorsal column in one of the marsupials (*Halmaurus giganteus*).

The rodents form a large order and comparatively few species have been examined, but here there is a distinct decussation of the pyramids in the lower part of the medulla oblongata, the crossed fibers passing into the dorsal columns. There is one notable exception, however, and that is the Leporidae, including the rabbits and hares. In this family the fibers, after decussating, pass into the lateral column. In the rabbit the crossing is not complete; a few fibers can be traced into the lateral column of the same side. The pyramid tract in this animal, therefore, appears to agree in every respect with that in the cat, dog, and monkey, except that its cortico-spinal fibers are more scanty.

In the rat the pyramid tract was found by Stieda ('69) to decussate into the posterior column, and this has been confirmed by Spitzka ('86), Goldstein ('03), Van der Vloet ('06), King ('10) and Ranson ('13). Most of these observers believe that the decussation is complete.

A complete crossing of the cortico-spinal fibers into the posterior column has also been described by Kotzenberg ('99) in the marmot, and by Bechterew ('90), Wallenberg (cited by Goldstein '03) and Reveley and Simpson ('09) in the guinea-pig.

In the Canadian porcupine (*Erethizon dorsatus*, Linn.) the condition is unique (Simpson '12). The fibers of the anterior pyramid, on entering the cord, divide into four fasciculi, two crossed and two direct. Of the crossed fibers, the greater number pass into the dorsal column (crossed dorsal tract), but a few can be followed into the lateral column (crossed lateral tract). A very considerable number of fibers remain uncrossed and are continued into the spinal cord, forming a comparatively large and compact bundle in the ventral column extending along

the margin of the ventral longitudinal fissure (direct ventral tract). Some fibers are also found in the dorsal column of the same side, forming a direct dorsal tract.

In the brief summary of previous work on the subject given above, no mention has been made of the methods employed, but in the great majority of cases these were faulty, consisting as they did in the examination of serial sections from normal histological preparations. Particularly has this procedure been adopted in the case of the smaller rodents and insectivores, where the difficulty of operating on the living animal is considerable.

In this relation it should be kept clearly in mind that only two methods are available for tracing the paths of fiber bundles in the central nervous system, namely, the embryological method of Flechsig and the Wallerian method of secondary degeneration. The latter, when followed by Marchi staining, is always to be preferred. As Edinger truly says "Ein echter Tractus cortico-spinalis ist nur durch Degenerationsversuche zu erkennen. Diese fehlen noch für allermeisten Säuger."

Granted that in serial sections through the decussation in normal preparations the fibers can be traced into one or other of the columns of the spinal cord, it is begging the question to say that these same fibers have their origin in the cerebral motor cortex. They may have some other source and may have joined the tract at a lower level, in which case they will not belong to the pyramidal system and will certainly not be cortico-spinal fibers. Again, where the degeneration method is not used, it is impossible to say whether the decussation is partial or complete. Take as an example the case of the cat, dog, or monkey, where there is a crossed lateral and also a direct lateral tract in the cord. When the cortical lesion is unilateral only one pyramidal system undergoes degeneration and at the decussation, if the Marchi method has been used, it is plainly seen that while most of the fibers decussate into the opposite lateral column, some pass into the corresponding column of the same side without crossing the raphé. If both pyramids were degenerated, or if the fibers were traced in normal preparations, the presence of this direct tract would

certainly be overlooked since it would be masked by the much larger crossed tract which occupies the same position in the cord.

Nowhere in the literature are to be found entirely satisfactory descriptions of the position of the pyramid tract in the brain and spinal cord in the lower orders of mammals. These are confined, for the most part, to the character of the decussation and do not deal with the topographical relationships at the different levels. It seemed desirable, therefore, to investigate further the course of this important tract by the Marchi method in such of the smaller rodents as could be obtained in this country, and the present paper gives the results as found in two closely allied species, namely, the red squirrel and the chipmunk.

PRESENT INVESTIGATION

In three red squirrels (*Sciurus hudsonius loquax*) and three chipmunks (*Tamias striatus lysteri*), the motor cortex of the left cerebral hemisphere was successfully removed, the operation, in each case being performed under ether anesthesia. After exposing the brain the motor area was located by electrical stimulation but it was found impossible to delimit the area with any degree of accuracy on account of the small size of the animal. It was difficult to get exposure close up to the middle line since the dura mater is very thin and the great longitudinal sinus easily ruptured. After the motor area had been located a shallow incision was made around it with a tenotomy knife and the cortex scraped away with a sharp spade within the limits of this incision. The part of the area extending along the margin of the longitudinal fissure was 'under cut' so as to divide all the nerve fibers coming from it but it was not entirely removed for the reason given above, namely, the risk of hemorrhage from the longitudinal sinus.

The animals were kept under observation for periods varying from thirteen to sixteen days after operation; they were then killed by coal gas, the brain and cord removed and placed in 3 per cent potassium bichromate solution. After hardening for three weeks in this solution, with frequent changing, the tissue

was cut into slices from 2 to 3 mm. thick and placed in Marchi's fluid made up according to the formula of Van Gehuchten ('06) as follows:

Osmic acid, 1 per cent.....	1 part
Potassium bichromate, 3 per cent.....	4 parts

In this solution they were allowed to remain for three weeks. Before being sliced up, photographs of the brain were taken, in each case, to show the position of the lesion.

In Marchi staining it is essential that a large excess of the fluid be used. The pieces of tissue while in the fluid, should be kept in tightly stoppered bottles in a dark and cool room in order to prevent the evaporation of the very volatile osmic acid and its decomposition by the action of light.

The original fluid employed by Marchi consisted of 1 per cent osmic acid one part, Muller's fluid two parts, and in this the tissue was kept for ten days after ten days previous hardening in Muller's fluid. It is claimed, however, that better penetration is secured by using the weaker solution and allowing it to act for a longer time; this I believe to be the case. Further, at the end of the staining process the fluid should not be thrown away; it may be used over and over again with the best results, all that is necessary being the addition of some fresh osmic acid and a small quantity of concentrated solution of potassium bichromate.

Several modifications of this method have been introduced from time to time, for example, by Orr ('00), Vassale ('96), Busch ('98) and others, but in my experience the original Marchi fluid, as used by Van Gehuchten, in its more dilute form, is better than any other. If due attention is paid to the details mentioned above, Marchi staining seldom fails to give good results.

The survival-time after operations is also an important consideration. According to Lange ('00) the optimum time comes between the tenth and fourteenth days. No doubt this will depend, to some extent, on the species of animal used. Most observers are inclined to allow a longer interval than fourteen days. Lange believes that the best results are obtained from adult animals. He fixes his tissue in a mixture of 10 per cent formalin

and Muller's fluid for the first two or three days and then transfers it to pure Muller's fluid or 3 per cent potassium bichromate solution, changing several times. He also prefers the stronger solution for staining—osmic acid one part, Muller's fluid two parts. In my experience formalin fixation gives uncertain results.

After removal from the Marchi fluid the pieces were washed for several hours in running water, then placed in 75 per cent alcohol, carried up the alcohol—xylene—paraffin series, and imbedded and cut in paraffin. In some cases celloidin was used as the imbedding material.

The entire brain from the mesencephalon to the lower end of the medulla oblongata, including the first cervical segment of the cord, was cut into serial sections, all of which were mounted. This gave series which were not quite continuous, however, since there was always some unavoidable loss in trimming the surfaces of the pieces into which the brain had been originally divided, prior to immersion in the Marchi fluid. Sections were also mounted from each segment of the spinal cord.

RESULTS OBTAINED

Red squirrel

Since the lesion was practically the same in all cases (fig. 1), it will be sufficient to describe in detail the results obtained in one individual of each species. As a matter of fact, it was found that the areas of degeneration at corresponding levels agreed closely in all the animals although some slight differences exist, particularly in the region of the decussation. All the figures refer to the same individual and were drawn from the preparations with the help of a Gage projection apparatus.

Transverse section of mesencephalon through anterior corpora quadrigemina (fig. 2). At the level of the exit of the third nerve the crusta shows degeneration over the entire area of its transverse section, but the lateral and mesial extremities contain fewer fibers than the middle portions. The gray matter of the substantia nigra contains many transversely cut fibers immediately

behind the crista; these do not appear to be running dorsalward into the tegmentum, as they are frequently observed to do in the cat and other animals at this level. Their course is cephalo-caudal, and they probably represent straggling bundles detached from the main mass of the tract.



Fig. 1 Brain of red squirrel, dorsal view, showing position of lesion in left cerebral hemisphere. Magnified $\frac{1}{4}$.

Transverse section near junction of mid-brain and pons through posterior corpora quadrigemina (fig. 3). The tract is now compacted into a single oval-shaped bundle lying just behind the superficial transverse fibers of the pons. Its long diameter extends from before backward and outward. This area contains a large number of degenerated fibers scattered uniformly over its entire surface; none can be seen leaving the bundle on any of its aspects.

Transverse section through middle of pons (fig. 4). The outline of the tract is still more or less oval with a slight projection at the inner angle. The bundle is single and compact, not broken up by the transverse pontine fibers as is the case in the cat, dog and monkey, for example; the black dots are very numerous and are scattered over the whole area. Fine degeneration is very abundant amongst the cells of the nuclei pontis on the mesial, ventral and lateral aspects of the tract; it fades away towards the middle line and does not extend beyond the raphé. No degenerated fibers are to be seen in the mesial fillet lying behind and internal to the pyramid tract.

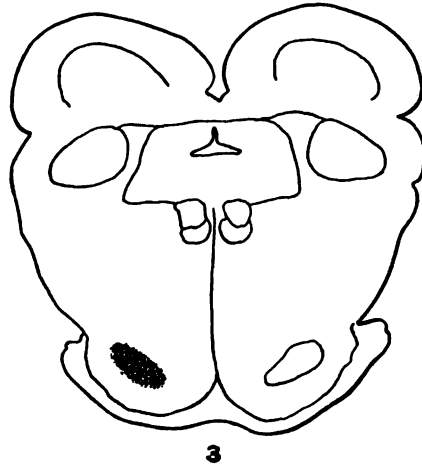
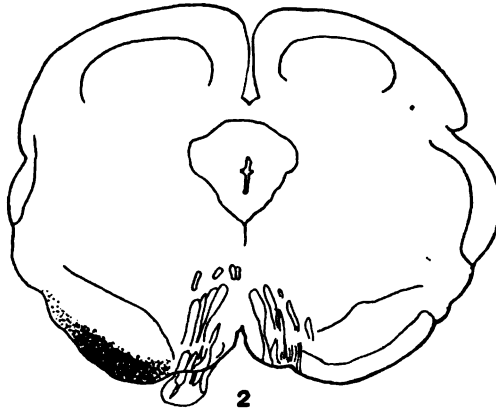


Fig. 2 Transverse section of mesencephalon through anterior corpora quadrigemina. $\times 6$

Fig. 3 Transverse section, near junction of mid-brain and pons. $\times 6$.

Transverse section through junction of pons and medulla oblongata (fig. 5). Sections passing through the seventh nucleus show that the tract has now reached the surface and entered into the formation of the anterior pyramid. Its transverse outline at this level is somewhat lens-shaped, the posterior surface being flattened towards the inner angle and showing an increased curvature towards the outer. The degeneration is abundant and is

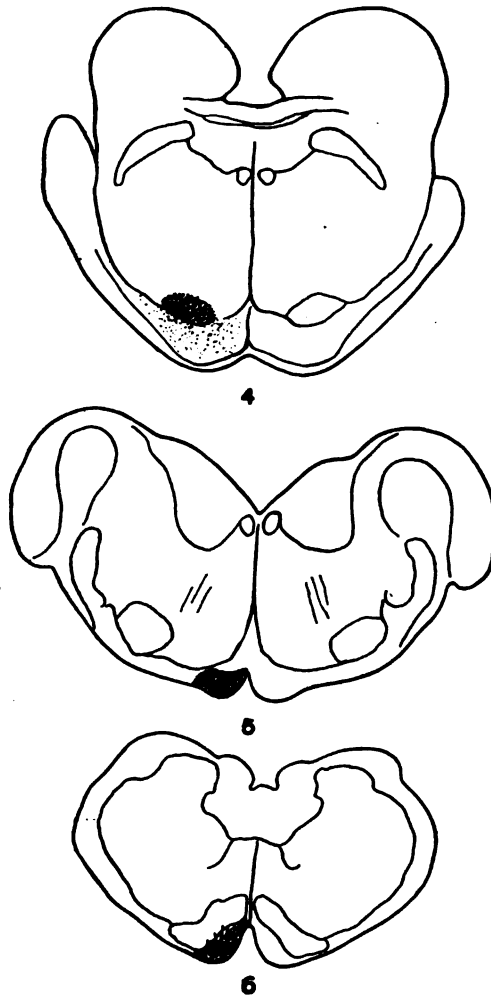


Fig. 4 Transverse section, through middle of pons. $\times 6$.

Fig. 5 Transverse section, near junction of pons and medulla oblongata. $\times 6$.

Fig. 6 Transverse section, medulla oblongata. $\times 6$.

scattered uniformly over the whole area. At this level a few fibers from about the middle of the posterior surface can be seen passing backward and inward towards the middle line; none of these, however, reach the raphé, nor can any be followed to the masses of gray matter in the neighborhood.

Transverse section through medulla oblongata (fig. 6). The section here described passes through the point where the central canal opens into the fourth ventricle. The pyramid has now a plano-convex outline the posterior surface being flattened against the inferior olivary nucleus which lies behind. The long axis runs from the middle line obliquely outward and forward. The whole area is studded with black dots and some fibers can be traced for a very short distance into the olivary nucleus but no indication of fine degeneration is visible in its gray matter. The inner angle of the pyramid is now becoming pointed and some fibers can be seen streaming out from this angle toward the median raphé but none cross as yet at this level.

Transverse section of medulla oblongata through middle of inferior olivary nucleus (fig. 7). The degenerated fibers coming off from the inner angle of the pyramid can now be followed in small bundles across the raphé. They are at first directed backward toward the posterior longitudinal fasciculi in front of the central canal and cross the raphé very obliquely. When almost in contact with these fasciculi they turn suddenly outward and then curve backward through the central gray matter entering the funiculus cuneatus in which they bend caudalward. In their passage through the gray matter they form bow-shaped curves with the concavities toward the central canal. At this level the transversely cut fibers in the funiculus cuneatus form a thin zone lying against the posterior aspect of the gray matter.

Transverse section of medulla oblongata through middle of pyramidal decussation (fig. 8). At a slightly lower level than that outlined in figure 7, the degenerated fibers are crossing in large numbers and follow practically the same course as already described in the last section. The pyramid itself is now considerably diminished in size and the area of degeneration in the funiculus cuneatus has broadened antero-posteriorly and also moved somewhat towards the middle line but it has not yet invaded the funiculus gracilis.

Transverse section of medulla oblongata through the lower end of inferior olivary nucleus (fig. 9). At this level the decussating fibers are much reduced in number and cross the raphé less obliquely

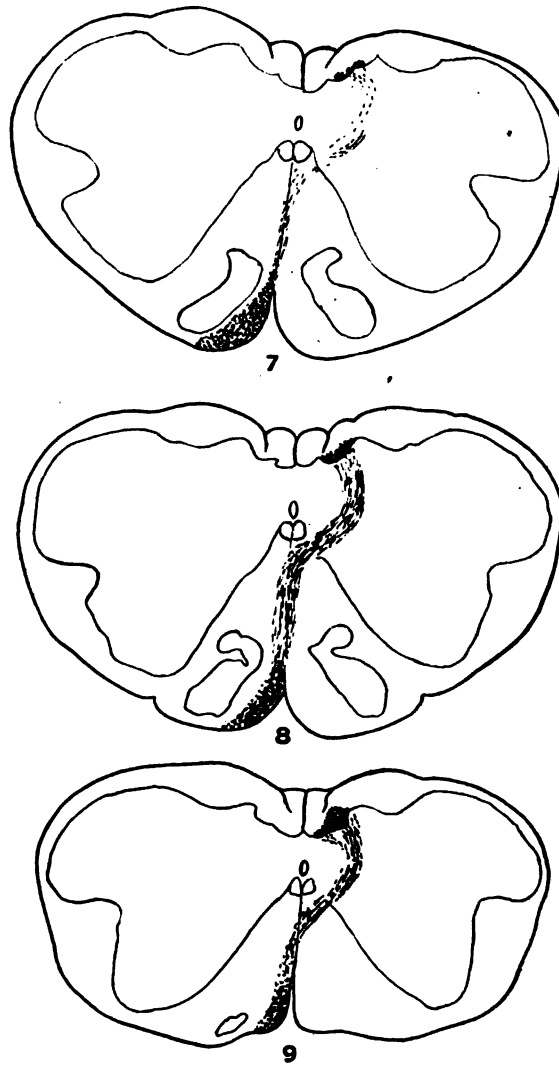


Fig. 7 Transverse section, medulla oblongata through middle of inferior olivary nucleus. $\times 12$.

Fig. 8 Transverse section, medulla oblongata through middle of pyramidal decussation. $\times 12$.

Fig. 9 Transverse section, medulla oblongata through lower end of inferior olivary nucleus. $\times 12$.

than formerly. The bundles interlace very distinctly with those of the normal pyramid. The pyramid is much reduced in size and the area of degeneration dorsal to the gray matter is correspondingly enlarged. The latter is somewhat triangular in outline and the innermost fibers lie nearer to the middle line but not within the limits of the funiculus gracilis. It will be observed that most of the decussation has taken place above the level of the lower end of the inferior olivary nucleus, and that it is very abrupt, the fibers running almost at right angles to the direction of the pyramid in their passage across the gray matter.

Transverse section through medulla oblongata about the level of the lower extremity of the pyramidal decussation (fig. 10). What remains of the pyramid is here seen as a narrow zone of degeneration bordering the ventral longitudinal fissure, but most of the fibers have crossed and are to be found in the posterior column where they form a large tract, the sharp inner angle of which reaches almost to the middle line.

Transverse section near junction of spinal cord and medulla oblongata (fig. 11). The decussation is now over and the crossed pyramid tract occupies a comparatively large area in the posterior or dorsal column, in contact with the posterior commissure and extending from the mesial septum laterally into the funiculus cuneatus. No crossed fibers at any level have been observed turning outward through the gray matter to reach the lateral column, nor have any been found to pass into the dorsal or lateral column of the same side. In this animal the decussation appears to be complete and the crossed pyramid tract is confined to the dorsal column.

Transverse section through first cervical segment of spinal cord (fig. 12). The pyramid tract in the cord occupies the same relative position as in the last section. The lateral diameter of the area is somewhat diminished and the antero-posterior diameter increased while the inner extremity is pressed up against the posterior median septum.

Transverse section through second cervical segment (fig. 13). The appearance of the tract is now greatly changed. Its outline is that of an isosceles triangle with the base lying against the

posterior commissure and the apex directed backward. As compared with that of the first cervical segment the area is somewhat diminished and the degeneration is less dens; from this it would seem that a considerable number of fibers have already disappeared from the tract.

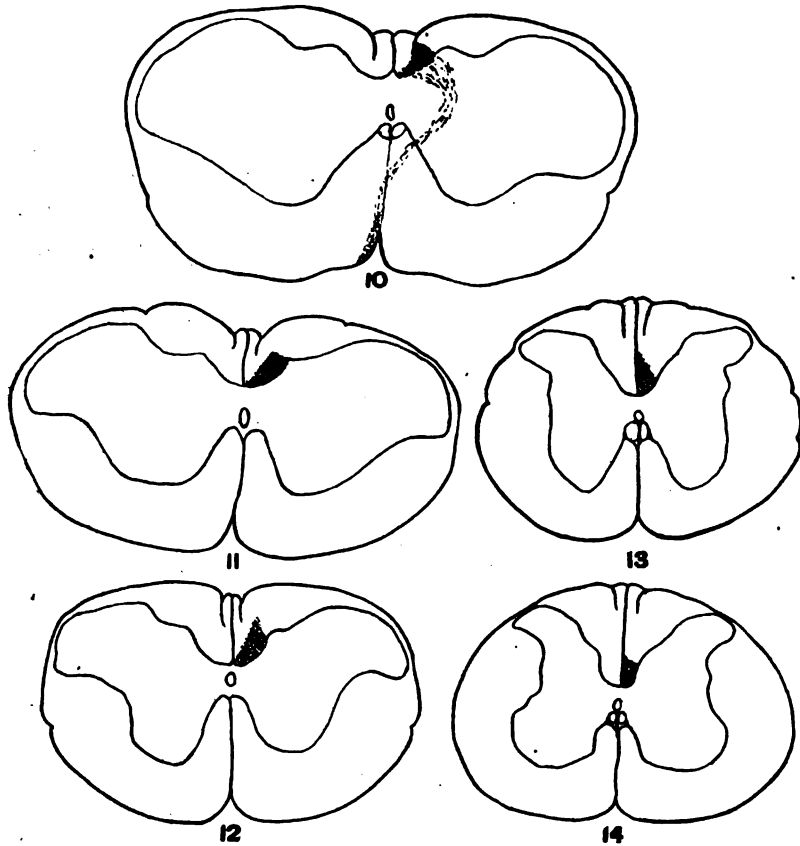


Fig. 10 Transverse section, medulla oblongata near lower limit of pyramidal decussation. $\times 12$.

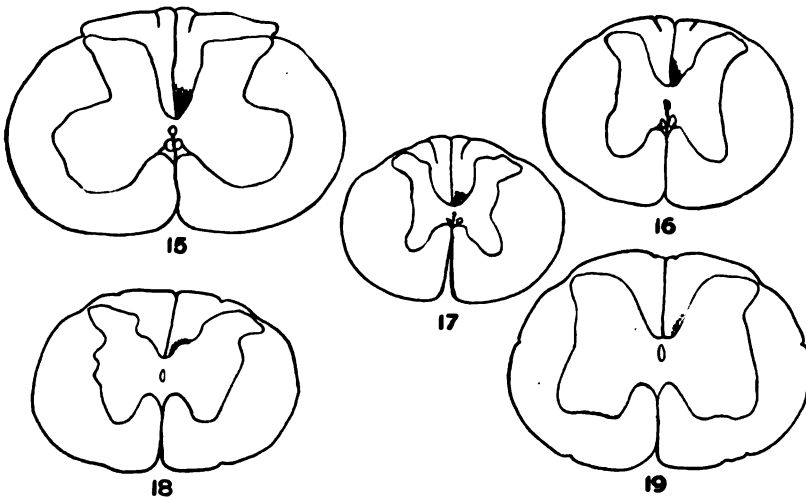
Fig. 11 Transverse section, near junction of medulla oblongata and spinal cord. $\times 12$.

Fig. 12 Transverse section, first cervical segment of spinal cord. $\times 12$.

Fig. 13 Transverse section, second cervical segment. $\times 12$.

Fig. 14 Transverse section, fourth cervical segment. $\times 12$.

Transverse section through fourth cervical segment (fig. 14). The tract now occupies the apex of the posterior column being squeezed in between the median septum and the base of the posterior horn. The outline is still triangular but the base at this level is directed backward and the apex forward while the mesial border is considerably shortened.



- Fig. 15 Transverse section, seventh cervical segment. $\times 12$.
Fig. 16 Transverse section, second thoracic segment. $\times 12$.
Fig. 17 Transverse section, eighth thoracic segment. $\times 12$.
Fig. 18 Transverse section, second lumbar segment. $\times 12$.
Fig. 19 Transverse section, fifth lumbar segment. $\times 12$.

Transverse section through seventh cervical segment (fig. 15). At this level there is little change; the tract is somewhat reduced in size and the degenerated fibers are less numerous.

Transverse section through second thoracic segment (fig. 16.) The outline of the tract here resembles that seen in the second cervical segment, being triangular in form with the base lying against the posterior commissure and the apex pointing backward. Between this and the seventh cervical segment a large number of fibers have disappeared.

Transverse section through eighth thoracic segment (fig. 17). At this level the tract is greatly reduced in size; it occupies a comparatively small area at the apex of the column.

Transverse section through second lumbar segment (fig. 18). The degenerated fibers are now found in close relation to the base of the posterior horn; they have left the middle line and extend along the mesial border of the gray matter, but no fibers have been observed entering the gray matter at this or any other level in the spinal cord.

Transverse section through fifth lumbar segment (fig. 19). All that represents the crossed pyramid tract here are a few scattered fibers lying close to the gray matter. In this particular animal the degeneration cannot be followed farther than this segment, but in one of the three squirrels used in these experiments some fibers are still present in the last sacral segment.

For the squirrel these results agree with those of Goldstein ('03) who found that the decussation in the lower part of the medulla oblongata is complete, all the fibers passing into the posterior columns. With regard to the course of the tract in the spinal cord he gives no details. He was chiefly concerned with the behavior of the fibers at the decussation. For this animal he was the first to use the degeneration method and so to prove that there does exist a cortico-spinal tract.



Fig. 20 Brain of chipmunk, dorsal view, showing position of lesion in left cerebral hemisphere. Magnified $\frac{1}{4}$.

Chipmunk

In the chipmunk the degeneration is similar to that found in the squirrel in the mid-brain, pons and medulla oblongata until the decussation is reached, so that it will be unnecessary to describe sections above this level. It is confined to the side of the lesion. Of the three animals used, one is selected for description but the results obtained in all are in close agreement. The lesion in the left cerebral hemisphere as it appeared in this animal when the brain was removed is shown in figure 20.

Transverse section through medulla oblongata near upper limit of decussation of pyramids (fig. 21). The pyramid, at this level, lies ventro-mesial to the inferior olivary nucleus. Its transverse section is oval in outline, pointed at the inner and outer extremities. From the inner angle a few fibers, cut longitudinally, can be seen passing toward the raphé but none as yet reach it; this represents the beginning of the decussation. The degeneration appears to be confined entirely to the left side, that is, to the side of the cortical lesion.

Transverse section of medulla oblongata through pyramidal decussation (fig. 22). The pyramid is still oval-shaped but the direction of its long axis is more dorso-ventral than in the last section. Degenerated fibers are seen crossing the raphé in bundles which interlace with those of the sound pyramid. They curve dorsolward through the gray matter and pass into the funiculus cuneatus in which they turn toward the spinal cord.

Transverse section of medulla oblongata through middle of pyramidal decussation (fig. 23). This section passes through the middle of the decussation; the inferior olivary nucleus is still seen. The degenerated fibers are crossing in large bundles and the pyramid is much reduced in size. A large crossed tract is now present in the funiculus cuneatus and it has moved nearer to the middle line. No fibers, at this or at any other level, turn outward in the gray matter toward the lateral column and none join the bundles of the sound pyramid and pass to the dorsal column of the same side, as they can be seen to do in the porcupine.

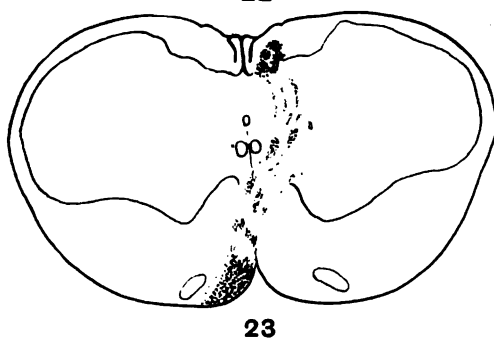
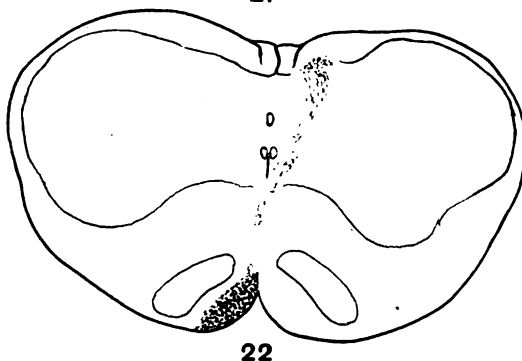
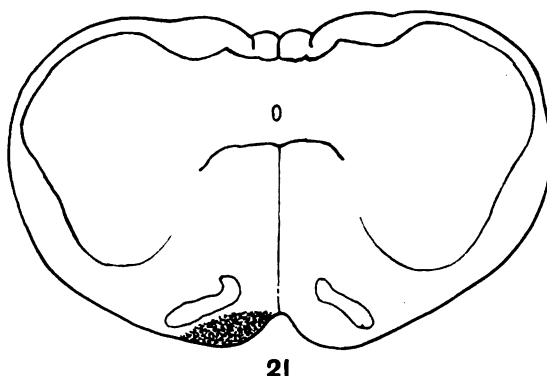


Fig. 21 Transverse section, medulla oblongata above decussation of pyramids. $\times 12$.

Fig. 22 Transverse section, medulla oblongata through pyramidal decussation. $\times 12$.

Fig. 23 Transverse section, medulla oblongata through pyramidal decussation. $\times 12$.

In the chipmunk, as in the squirrel, the decussation appears to be complete.

Transverse section through medulla oblongata just caudal to pyramidal decussation (fig. 24). All the fibers have now crossed and are found in the posterior column cut transversely, where they form the dorsal pyramid tract. It is larger than in the previous sections and has moved nearer to the middle line. At its inner angle two distinct bundles are seen in this section.

Transverse section through cephalic portion of first cervical segment of the spinal cord (fig. 25). This section, passing through the proximal part of the first cervical segment, shows the crossed dorsal tract lying in the posterior column. It is wedge-shaped in outline, with the base lying against the posterior commissure and the apex directed dorsalward. The inner angle now reaches the posterior median septum.

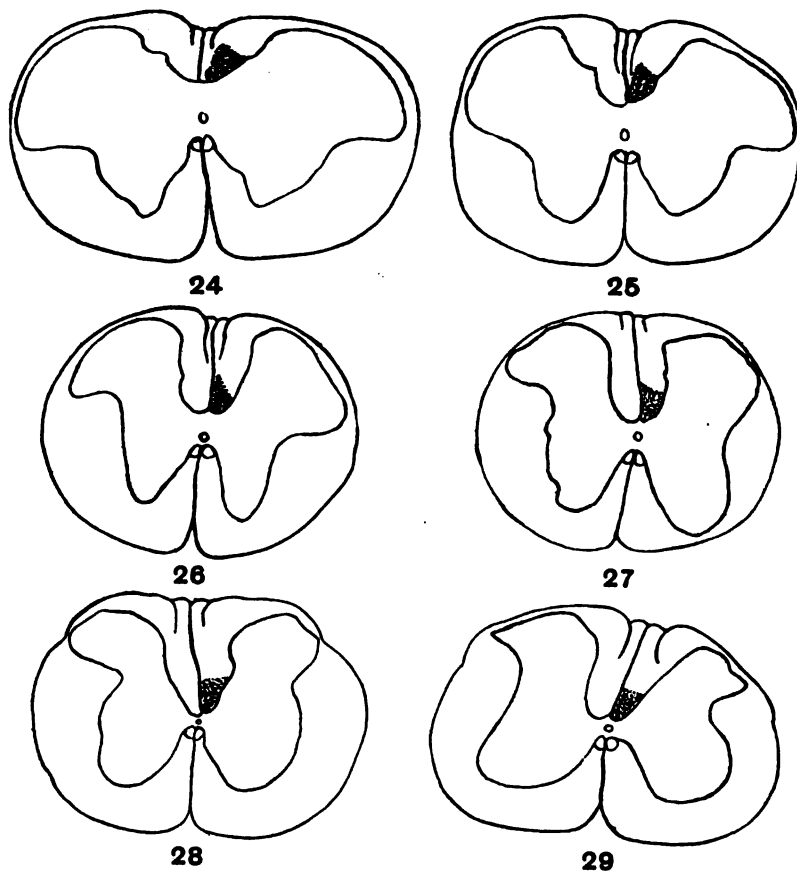
Transverse section through caudal portion of first cervical segment (fig. 26). In the first cervical segment the tract undergoes rapid changes in size and form. Its area in the caudal portion of the segment is somewhat reduced and the degenerated fibers which it contains are not so closely packed as in the last section. It is still triangular in shape but it is narrowed laterally and almost the whole extent of its mesial border lies in contact with the posterior median septum.

Transverse section through second cervical segment (fig. 27). The tract now lies in the angle formed by the median septum with the border of the posterior horn. It is still triangular in outline but the base is directed backwards and the apex forwards.

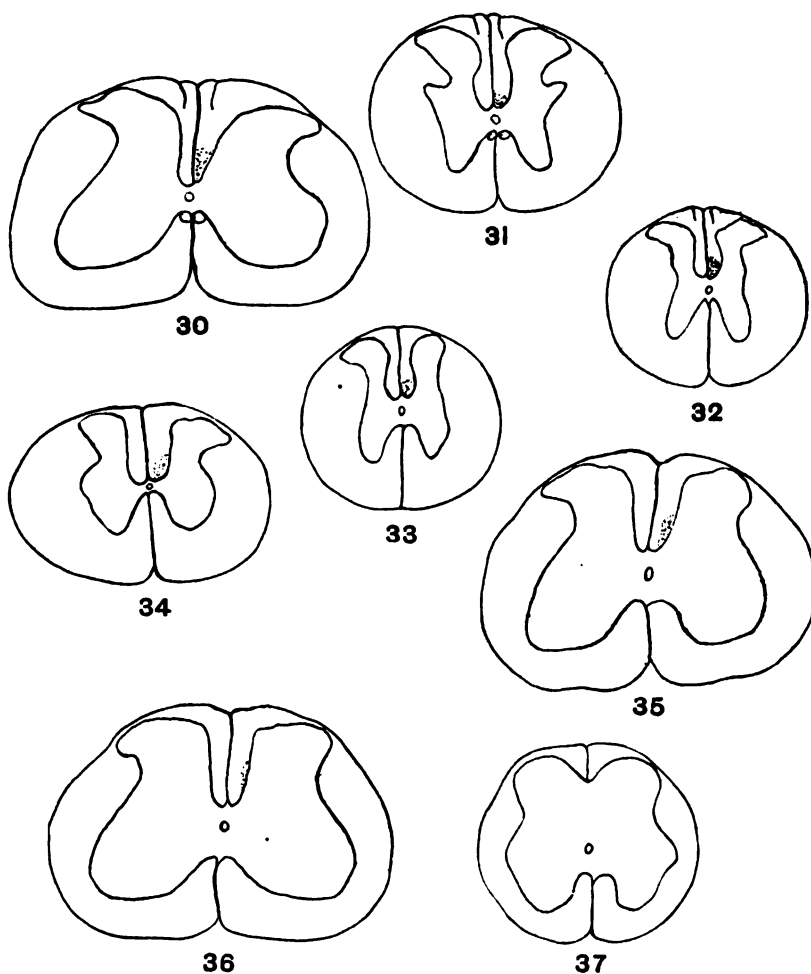
Transverse section through fourth, sixth and eighth cervical segments (figs. 28, 29, 30). At these three levels there is little change except in the number of degenerated fibers, which decrease as the thoracic region is approached. The area occupied by the tract in each of these segments is shown in the corresponding figure.

Transverse section through second thoracic segment (fig. 31). The tract is considerably reduced in size, but shows the same general outline and occupies the same position as in the cervical region.

Transverse section through seventh thoracic segment (fig. 32). In the mid-thoracic region the tract has the same general outline as in the second thoracic segment, but it extends farther dorsalward along the median septum. There is also some reduction in the number of degenerated fibers which it contains,



- Fig. 24 Transverse section, medulla oblongata. $\times 12$.
Fig. 25 Transverse section, upper level of first cervical segment of cord. $\times 12$.
Fig. 26 Transverse section, first cervical segment, lower level. $\times 12$.
Fig. 27 Transverse section, second cervical segment. $\times 12$.
Fig. 28 Transverse section, fourth cervical segment. $\times 12$.
Fig. 29 Transverse section, sixth cervical segment. $\times 12$.



- Fig. 30 Transverse section, eighth cervical segment. $\times 12$.
Fig. 31 Transverse section, second thoracic segment. $\times 12$.
Fig. 32 Transverse section, seventh thoracic segment. $\times 12$.
Fig. 33 Transverse section, twelfth thoracic segment. $\times 12$.
Fig. 34 Transverse section, first lumbar segment. $\times 12$.
Fig. 35 Transverse section, third lumbar segment. $\times 12$.
Fig. 36 Transverse section, first sacral segment. $\times 12$.
Fig. 37 Transverse section, fourth sacral segment. $\times 12$.

Transverse sections through twelfth thoracic and first lumbar segments (figs. 34 and 35). In the twelfth thoracic segment the tract still meets its fellow of the opposite side at the posterior median septum but in the first lumbar segment this is no longer the case; it has now left the septum and is separated from it by a zone of sound fibers, the degenerated area lying against the mesial border of the posterior horn.

Transverse sections through third lumbar and first sacral segments (figs. 35 and 36). At the level of the third lumbar segment the degenerated fibers are greatly reduced in number and form a narrow zone along the ventral part of the mesial border of the posterior horn. In the first sacral segment very few fibers remain.

Transverse section through fourth sacral segment (fig. 37). At this level six or eight degenerated fibers can still be seen in the posterior column.

Comparing the figures at corresponding levels in the medulla oblongata and spinal cord, it will be seen that the distribution of the pyramid tract in the squirrel and chipmunk agrees closely. In both species it is confined to the dorsal column in the spinal cord and there is no trace of a direct tract.

SUMMARY

The cortical motor areas in the left cerebral hemisphere were extirpated in three red squirrels (*Sciurus hudsonius loquax*) and three chipmunks (*Tamias striatus lysteri*) and the resulting degeneration followed by the Marchi method.

The pyramid tract occupies the usual position in the crista, pons and medulla oblongata until the decussation is reached. Here, in the lower part of the bulb, the fibers cross the middle line abruptly in bundles which interlace with those of the sound side and pass through the gray matter into the funiculus cuneatus where they turn caudalwards to enter the spinal cord and form the crossed pyramid tract. The decussation is complete, no fibers remaining on the same side when the spinal cord is reached.

There is no sign of a crossed lateral tract.

The dorsal tract can be followed as far as the lower sacral segments.

A considerable number of degenerated fibers disappear in the upper cervical segments of the spinal cord.

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CORRELATED ANATOMICAL AND PHYSIOLOGICAL STUDIES OF THE GROWTH OF THE NERVOUS SYSTEM OF AMPHIBIA

I. THE AFFERENT SYSTEM OF THE TRUNK OF AMBLYSTOMA

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SIXTY FIGURES

The investigations upon the basis of which this paper is written have been in progress for several years. My purpose in the work and my general plan of study of the relation between the development of particular structures of the nervous system and the behavior of the embryo have been stated in several publications. In brief, my effort has been to analyze the function of the receptor system, to determine whether the development of movements is in a regular order or haphazard, to discover the exact relation of the nature of the stimulus to reaction, and to study the relation of behavior to the processes of growth and differentiation throughout the nervous system. Through such studies it has been expected that new light may be thrown upon the function of particular parts of the nervous system, the causal factors in behavior, elementary processes in the action of the nervous system and possibly upon fundamental problems of growth. That knowledge from this source may prove of interest to psychology also, is perhaps not too much to anticipate.

My earlier results were read, in part, before the American Society of Zoologists and the American Association of Anatomists in their Chicago meetings of 1907, and published a little later in the *Anatomical Record* (July, 1908) and in this *Journal* (June, 1909). These communications described the movements

of embryos of *Diemyctylus torosus* from the earliest responses to tactile stimulation up to the time when the embryo can swim, and showed that there are distinct types of movement which develop in a regular order of sequence till they culminate in locomotion. Later results from studies chiefly upon *Amblystoma* were presented before the American Association of Anatomists in the Cleveland meeting of 1912 and before the American Philosophical Society in Philadelphia, April, 1913. The part of the former report that related particularly to the motor column of the spinal cord was published in this Journal, April, 1913; the report before the American Philosophical Society is abstracted in *Science*, May 9, 1913 (p. 722). In these communications it has been emphasized that the earlier responses of the embryos under consideration are determined by the nature of the primary, or first reflex arc that becomes demonstrable by histological methods, that the progressive development of this arc determines the order of development of somatic movements and that the final common path of the reflexes stimulated from the various fields as the embryo develops becomes the nervous mechanism of locomotion.

These earlier presentations of my work have been made as preliminary to a more exhaustive treatment of the growth processes in the nervous system in their relation to behavior, and it is, accordingly, my purpose to make this communication one of a series which will treat in a more complete manner the particular phases of the problem in hand. As the first of such a series, this paper deals with the development of the sensory system of the trunk in its relation to the behavior of embryos of *Amblystoma* up to the swimming stage. The species used have been *A. punctatum* and *A. opacum*, but the latter only rarely.

I. THE ANATOMICAL PART

Embryos have been selected for this study according to the physiological standards described in my earlier papers, namely: (1) Embryos that move in response to an electric current but not in response to tactile stimulation with a hair or similar non-conducting and chemically inactive structure, designated as non-motile stage; (2) Embryos taken very soon after there is perceptible movement in response to tactile stimulation, designated early-flexure stage; (3) Embryos that move the trunk into a coiled condition, designated the coiled-reaction stage; (4) Embryos that move to the right and left simultaneously in different parts in a sinuous fashion, designated the S-reaction stage; (5) Embryos that have just acquired the power of locomotion through the serial S-reaction movements, designated the early-swimming stage.

For this study of the sensory system the S-reaction stage has been omitted from the series. This omission has been made because the important differences between the S-reaction and the early-swimming embryos relate to the commissural and motor systems rather than to the sensory system, and do not offer any distinctive contribution to the phase of the problem which this paper treats.

The histological methods employed in the preparation of the material for study are noted in connection with the descriptions of the several figures.

1. THE GIANT GANGLION CELLS

Since there are no perceptible dorsal roots from the fundaments of the spinal ganglia during the period under consideration, the giant ganglion, or Rohon-Beard cells obviously constitute the sensory system of the trunk at this time. It is with these cells, therefore, that this paper preeminently deals.

This series of neurones, which appears very early in the dorsal portion of the spinal cord of aquatic vertebrates, has been the subject of study on the part of anatomists from an early period

of comparative neurology, chiefly from the purely morphological point of view. Questions of homology, however, have no critical consideration in this paper. It is the relation of the giant ganglion cells to a functioning and at the same time differentiating nervous system that is here the center of interest. It is not the intention, however, to ignore the anatomical results of earlier authors on the subject, and a consideration of their results will be given farther on in the paper from the point of view of my own findings, both anatomical and physiological.

The following anatomical observations have been made by exhaustive study with the oil immersion lens systems (Zeiss and Bausch and Lomb) of serial sections of fifty-nine embryos selected from a collection of over two hundred such series because of their special adaptability to this phase of the problem. Of this number, thirteen are of the non-motile stage; seventeen, of the early-flexure stage; fourteen, of the coil-stage; ten, of the early-swimming stage. As representative of these neurones in the embryos of the non-motile stage over two hundred of the giant ganglion cells and fibers have been studied minutely in their various relations; of the early-flexure stage, about the same number; of the coil-reaction stage, approximately one hundred; and about an equal number of the early-swimming stage.

a. The distribution of the cells

The distribution of the giant ganglion cells has been studied exhaustively in one type specimen of each of the four stages of development, and their positions have been charted in graphic projections on the vertical plane as figures 56 to 59 of this paper. Exact counts have been made of the cells in these four cases only, but my study of numerous embryos convinces me that the number of the cells and their manner of distribution is fairly constant for each stage and that these features of the system, with minor qualifications as noted farther on, are typically represented in the figures cited.

The cells charted in the figures 56 to 59 are on one side of the median plane only, and, therefore, represent presumably about

one-half the number of the whole system in the spinal cord. By actual count there are on one side of the non-motile embryo 210 of these cells; in the embryo of the early-flexure stage, 222; in the embryo of the coil stage, 197; in the embryo of the early-swimming embryo, 253.

Except for the lower number in the coil stage there would appear in this series a gradual and progressive increase in the number of cells during the period under investigation. Indeed, such an increase in clearly differentiated cells probably occurs, for it is my judgment that the reduced number observed in the embryo of the coiled-reaction stage is due to the fact that the staining of this specimen is not well adapted to the differentiation of these cells from others around them. This is a misfortune from the point of view of this paper, and it should be mentioned that the four typical embryos were not selected for the projection of this system of neurones alone. They were selected upon more general considerations and the projections were made for the general purposes of the more comprehensive study that is in progress. The giant ganglion cells have simply been sketched into the projected outlines of the nervous and muscular systems which are being used for other phases of the work.

In the distribution according to regions it is found that within the range of the first eighteen post-auditory myotomes, which may be regarded as constituting the trunk, there are 149 giant-ganglion cells in the non-motile embryo; 150, in the early-flexure embryo; 128, in the coiled specimen; 176, in the embryo of the swimming stage. Caudad of this there are 61 in the non-motile; 72, in the early-flexure; 69, in the coil stage; 77, in the early-swimming stage.

Too great meaning should not be attached to the numerical details of these counts for, while the cells were counted with great care, the specimens were not prepared with a view to equivalent differential staining of these elements in all cases. It happens, however, that the youngest and oldest members of the series received essentially the same fixation and staining, and may, therefore, be regarded as of equal value for making satisfactory counts. The numbers of cells found, in these two specimens may

be regarded as trustworthy and of positive value. The other two specimens may be regarded as corroborative evidence with reference to the count and as valuable illustrations of the changes that are taking place in the distribution of the giant ganglion cells, particularly in the tail region, during this period of growth.

It is obvious from a glance at the graphic projections that there is in the youngest embryo of the series a quite regular distribution of the giant ganglion cells throughout the trunk region, excepting through the level of the first three or four myotomes. In the caudal region, on the other hand, they are thickly congregated throughout the extent of the segmented mesoderm and then more scattered, caudad. In comparison with this latter feature, the embryo of the early-flexure stage shows a more general scattering of the neurones through the cord of the lengthening tail bud, and this same process of distribution is seen in the two later stages. In the embryo of the coiled-stage the distribution is quite regular through the cord caudad to the level of the twenty-ninth myotome. This is the case, also, in the embryo of the early-swimming stage. Caudad of this in the coiled stage the cells are widely scattered through the level of the segmented mesoderm. In the embryo of the swimming stage, on the other hand, the cells are not distributed through the entire extent of the segmented mesoderm.

It seems obvious from this study of the numbers and positions of the giant ganglion cells that there can be little if any proliferation of cells belonging to this series during the period under consideration, and that the distribution of the cells through the caudal portion of the cord is brought about chiefly or wholly through mechanical processes allied with the differentiation of the mesoderm, and after the cells of the system have become differentiated into neuroblasts and neurones. The slight increase in numbers from 210 to 253 on one side should be regarded as due, not to proliferation of cells of the group, but to a progressive differentiation which enables the observer to recognize more of the cells with certainty. More detailed evidence on this point will be given in following paragraphs.

Another noteworthy feature in the distribution of the giant ganglion cells is conspicuous in these projections, namely, that the cells diminish in numbers rapidly from the level of about the fourth post-auditory myotome rostrad and assume a more ventral position in the spinal cord, while in the more caudal regions, also, there is a marked shifting of the cells ventrad. This dorso-ventral position of the cells in the rostral region might be explained as due to the proliferation of other cells dorsally, in relation to the development of the nuclei of the dorsal funiculi and allied structures; but no such explanation can apply to the ventral position of the cells in the caudal parts of the cord. In the latter region the ventral position of the cells is distinctly allied with the position of mesoderm, as the following description of cross sections will show. In fact, when the entire spinal cord is taken into account, the dorso-ventral position of the giant ganglion cells shows a more distinct correlation with the position of the mesoderm than it does with any structures in the cord. In following the further descriptions, one should keep this point in mind.

b. The giant ganglion cells as a sensory column

For the purpose of illustrating the relations of the giant ganglion cells within the spinal cord as a sensory column and the processes of differentiation that can be recognized in the system during this period of development figures 28 to 54 have been made with the aid of a drawing apparatus at a magnification of four hundred diameters (reduced to two hundred in the figures). This series of drawings is intended for use in future descriptions of the growth of the spinal cord and with this in view the other structures in the sections have been drawn with as much care as has been given to the giant ganglion cells. No detailed description of the other features of the cord will be given here, but a cursory examination of the figures will enable one to appreciate somewhat the setting of the functional sensory column in the midst of a mass of rapidly proliferating and differentiating cells which belong to other groups.

As introductory to the study of cross-section drawings of smaller magnification it will be helpful to examine figure 27, which is drawn at a magnification of 940 diameters. It is taken from the level between fifth and sixth myotomes of an embryo of the early-swimming stage. The unipolar condition of the giant ganglion cell in the latero-dorsal region is clearly brought out in relation to the sensory tract and the peripheral fiber. The latter projects out into the myoseptum. In the latero-ventral region motor neurones may be recognized, and, farther dorsad, a group of commissural or associative neuroblasts. There is no perceptible commissure at this level. In the latero-ventral angle of the cord is seen the motor tract, which is differentiated from the sensory tract by its more open or vesicular condition. This is probably due to its being composed of larger fibers than those of the sensory tract. The sensory tract is composed of a thin sheet of fibers situated immediately against the external limiting membrane of the cord and extending from the dorsal border of the motor tract to the process of the giant ganglion cell. Reference to figure 27a will show the dendritic nature of the peripheral process of the giant ganglion cells, since the contents of the perikaryon, the yolk spherules, are found distributed out into the fiber a considerable distance beyond the limits of the cord. Many more pronounced illustrations of this condition are found in my preparations.

Figures 28 to 54, now to be described, are taken from the same specimens from which the projections of figures 56 to 59 were made, and should be studied in connection with these projections. They should, also, be studied in groups according to the following considerations: figures 28 to 31 are from the level of the third myotome of the several specimens; figures 32 to 35, from the level of the eighth myotome; figures 36 to 39, from the level of the thirteenth myotome; figures 40 to 43 from the level of the eighteenth myotome; figures 44 to 47, from the level of the twenty-third myotome; figures 48 to 50 from the level of the twenty-eighth myotome of the three oldest embryos; figures 51 to 53, from the level of the thirty-third myotome of the same specimens; figure 54, from the level of the unsegmented

mesoderm immediately caudad of the thirty-seventh myotome of the oldest specimen. Figure 51, however, is in reality taken from the level of the unsegmented mesoderm immediately caudad of the thirty-second myotome. Grouped according to specimens, figures 28, 32, 36, 40 and 44 represent the embryo of the non-motile stage; figures 29, 33, 37, 41, 45, 48, and 51, the early-flexure stage; figures 30, 34, 38, 42, 46, 49, 52, the coil-reaction stage; figures 31, 35, 39, 43, 47, 50, 53 and 54, the early-swimming stage.

(1) *At the level of the third myotome.* In figure 28, which is taken from the level of the third myotome of the non-motile embryo two giant ganglion cells are found in the most lateral portion of the cord midway from its dorsal to its ventral limits. These cells are filled with yolk spherules (not represented in the figures) as are all the cells of this group in this stage. A granular pigmentation outlines the margins of the cells with their ventrally projecting processes. In contrast with the surrounding cells, the nuclear plasma of the giant ganglion cells takes on a faint tinge of the color used for counterstain of the cytoplasmic elements and the chromatin has a finer structure. In the latter part of the period under consideration the nuclei of motor and commissural cells take on these same characteristics in a considerable degree, but in this early period of development the giant ganglion cells stand out as clearly the most differentiated cells of the spinal cord. The processes of the giant ganglion cells at this level in the youngest embryo are directed ventral for a short distance, but it is impossible to detect long fibers here or the presence of longitudinal fibers of the system, either in transverse or longitudinal sections.

In the corresponding level of the early-flexure stage, represented in figure 29, the giant ganglion cell appears in a little more dorsal position. Although no fiber tract can be seen in relation with these cells in cross sections here, longitudinal sections show spindle-shaped processes projecting from the giant ganglion cells cephalad and caudad, forming a continuous column without a differentiated fiber tract. Such a fiber tract becomes perceptible,

however, at this level in the coiled-reaction stage (fig. 30). It is made up of a few fibers that are scattered along for some distance ventrad from the cells and immediately against the external limiting membrane of the cord. These fibers are barely perceptible in cross-section. The more dorsally situated have a perceptible inclination ventrad when viewed in the cross section of the cord and the ventrally projecting processes of the giant ganglion cells are in close relation with them. The more ventrally situated fibers, however, are cut in distinct cross section and form a differentiated longitudinal fiber tract.

At the level of the third myotome in the early-swimming embryo the sensory tract has extended farther ventrad. In fact it reaches almost or quite to the motor tract (*VT*) which occupies the most latero-ventral portion of the cord. In its dorso-ventral extension, however, the sensory tract is interrupted here and there by the neuroblasts of other groups. The giant ganglion cells in this case also lie dorsally of the tract and send their processes into its dorsal portion.

(2) *At the level of the eighth myotome.* In the non-motile embryo, at the level of the eighth myotome, the giant ganglion cells are situated in the dorso-lateral portion of the cord (fig. 32). In the cross-sections there are still no perceptible fibers associated with them. In suitable plane of section, however, a few fibers are demonstrable in a position just ventral of the cells. The direction of these fibers is rostrad and ventrad from the cells and they are obviously attenuated processes from these cells. Although not perceptible in transverse section their position has been sketched in on figure 32.

As compared with the non-motile embryo, that of the early-flexure stage shows a marked development of the sensory tract at this level (fig. 33). The characteristic process of the giant ganglion cell is here seen projecting ventrad towards the tract, the fibers of which are scattered along against the limiting membrane to near the motor tract. Study of sections adjacent to the one figured shows that the fibers are distributed sparsely through the region and that cells push in between them and

against the limiting membrane of the cord. In the section from which figure 33 was taken only the fibers figured nearest the giant ganglion cell could be detected. Those pictured farther ventrad in the figure were sketched in from nearby sections of the same series.

In the embryo of the coiled-reaction stage (fig. 34) the sensory tract is perceptibly strengthened as compared with that in figure 33. It appears as a continuous sheet of fibers from the motor tract dorsad to the giant ganglion cells, which are in this stage, also, located dorso-laterally. Just dorsad of the motor tract the sensory tract narrows down and disappears. In the early-swimming embryo (fig. 35) the sensory tract is difficult to outline clearly in this particular section on account of the neuroblasts which have invaded its territory, but in the opposite side of the cord in this section and in near-by sections of the series the sensory tract shows the same general relations as in figure 34, except that it is more strongly developed in the older embryo. The process of the giant ganglion cell in the figure reaches to the dorsal border of the sensory tract.

(3) *At the level of the thirteenth myotome.* At the level of the thirteenth myotome the giant ganglion cells still occupy a dorso-lateral position in the cord of all four embryos (figs. 36, 37, 38, 39), and their processes extend towards or into the tract. In transverse section no distinct fiber tract can be detected at this level in the non-motile embryo, but in favorable planes of section spindle-shaped and attenuated processes can be traced from the giant ganglion cells into an indefinite tract of short fibers. Its position is indicated in figure 36. In the embryo of the early-flexure stage (fig. 37) a giant ganglion cell appears in the lateral region of the cord, midway from the dorsal to the ventral borders. No sensory tract can be clearly differentiated in transverse section. In embryos of the coiled-reaction stage also no sensory tract can be detected in transverse sections. In favorable planes of section, however, there is unmistakable evidence of a small tract of fibers in the position indicated in figure 38.

In the early-swimming embryo the sensory tract appears clearly in cross section as shown in figure 39. It extends here from just dorsad of the motor tract to the ventral projections of the giant ganglion cells.

(4) *At the level of the eighteenth myotome.* At the level of the eighteenth myotome in the non-motile embryo the spinal cord frays out dorsally into neural crest structures (fig. 40). Scattered from the dorso-lateral angle of the cord ventrad to the level of the dorsal margin of the myotome are several of the giant ganglion cells, which are conspicuously differentiated from the other cells of the vicinity. In longitudinal sections cells in this position are found to have a definite orientation with their long axis longitudinal in the cord. Almost the entire cord, therefore, at this level is sensory. Some of the ganglion cells are in proximity to the skin, while others are quite as intimately related to the myotomes. These features of the sensory column will be brought out in detail in the latter part of the description.

Comparison of figure 41, which is at the corresponding level of the embryo of the early-flexure stage, with figure 40 shows a great change in the general features of the cord and in the position of the giant ganglion cells. The sensory column here occupies the dorsal half of the cord. No sensory fibers can be detected in it by the study of cross sections, but in longitudinal sections, a distinct fiber tract appears at the ventral end of the ganglion cells. Its position is sketched in the figure. The fibers of this tract must be short, however, for if they had great length they would be found farther ventrad in accordance with the relations in the more rostral portions of the cord.

In the corresponding level of the coiled-reaction stage there is still no perceptible fiber tract, but, again, in suitable plane there appears a sensory tract which is more clearly differentiated than the motor tract at the same level (fig. 42). In the embryo of the swimming stage the sensory column still occupies the dorsal half of the cord, and a giant ganglion cell is seen far ventrad (fig. 43). The sensory tract here is more clearly differentiated than in the younger embryo.

(5) *At the level of the twenty-third myotome.* In considering figure 44 one should note that, owing to the ventral curvature of the caudal region (fig. 56), this section is somewhat oblique and that the dorso-ventral dimension is therefore magnified somewhat out of proportion to the width. The extreme ventral position of the giant ganglion cells in the caudal region, and their proximity to the myotomes as well as to the skin is demonstrated here. Only two such cells appear in this figure but adjacent sections show that their distribution is general from the latero-dorsal angle to the latero-ventral angle throughout the caudal portion of the cord in this age. A comparison of figures 56, 40, 44, 19, 20, 21 and 22 will help to establish a clear idea of the exact relations in this part of the embryo.

In this level of the embryo of the early-reaction stage the ganglion cells are still found in the dorsal half of the cord with their processes extending ventrad. While no fibers can be seen here in cross section, there is in longitudinal section a perceptible tract that is made up of spindle-shaped processes of the ganglion cells. The sensory column is essentially of this same composition in the coiled-reaction and early-swimming embryos (figs. 46, 47), that is to say, it is made up of spindle-shaped processes of the cells and a few fibers that can be detected only in longitudinal section.

(6) *The sensory column at the level of the twenty-eighth myotome.* The non-motile embryo drops out of consideration here since it has only twenty-three well differentiated myotomes. In the early-flexure stage at this level the myotomes have assumed a ventral position relative to the spinal cord and the giant ganglion cells are accordingly found far ventrad in immediate proximity to the myotomes. Other sections show them distributed dorsally from this level to the dorso-lateral angle of the cord, though none appears in that region in the figure (48). The same features of distribution begin to appear, also, in the embryo of the coiled-reaction stage (fig. 49), but in the early-swimming stage the sensory column appears again in the dorsal half of the cord (fig. 50). Longitudinal sections show that in all these cases

the cells are oriented with their long axis longitudinal in the cord.

(7) *At the level of the thirty-third myotome.* Figure 51, as stated above, is drawn through the unsegmented mesoderm just caudad of the thirty-second myotome in the embryo of the early-flexure stage. The ventral half of the cord here is purely epithelial in structure and the myotomes are situated ventrally with reference to the more differentiated part of the cord. The ganglion cells are closely related to the mesoderm as well as to the skin.

In the embryo of the coiled-reaction stage (fig. 52) the sensory column consists of spindle-shaped cells occupying the dorsal half of the cord, with their long axis longitudinally oriented. The dorsal part of the cord here frays out into neural crest elements. In the embryo of the early-swimming stage (fig. 53) the attenuation of the spinal cord at the level of the thirty-third myotome is noteworthy. The sensory column in this stage, also, occupies the dorsal portion of the cord. The myotomes here extend well dorsad, but in figure 54, which is taken from the level of the unsegmented mesoderm just caudad of the thirty-seventh myotome, the mesoderm is found far ventrad. The cord here is much wider than it is five myotomes cephalad. In fact, a lateral distention of the spinal cord at the tip appears to be typical, and in many cases, particularly in the younger embryos, there is a perceptible tendency for the central canal to form ventricular evaginations. This condition may simply be the result of a stress upon the sides of the spinal cord exerted by the mesoderm in its growth, for, as shown in later descriptions, there is a region of firm attachment between the neural tube and the mesoderm in the caudal region during the early periods of development.

(8) *Generalization upon the basis of these descriptions.* The foregoing description of the sensory column at arbitrarily selected levels has been employed as a means of presenting briefly the results of exhaustive study of serial sections in various planes, and it is hoped, demonstrates sufficiently the basis for certain generalizations concerning the nature and differentiation of this

part of the spinal cord during the period under investigation. These generalizations may be stated as follows:

1. The giant ganglion cells and their processes constitute the sensory column of the cord and are the source of all the fibers of the sensory tract. (In the rostral region of the tract of the older stages there may be axones of the descending trigeminal tract, but this question will be critically considered in another paper).

2. In the more caudal portion of the several ages the column consists of bipolar cells oriented longitudinally in the cord.

3. In the youngest embryo the same condition prevails in the most rostral portion of the column, but in the older stages the fibers seem to incline more ventrad.

4. The axones of the cells are directed cephalad into the tract and those arising from more caudally situated neurones assume a ventral position with reference to the axones of cells situated farther cephalad, the tract during this time being a thin sheet of fibers immediately against the external limiting membrane of the cord, intercepted here and there in its dorso-ventral extent by neuroblasts of other groups.

5. The cells of the column have dendritic processes directed caudad, so that the tract is adapted to conduction rostrad.

6. There is progressive differentiation in the column, consisting chiefly in the growth of axones cephalad.

7. At the beginning of the period the cells of the column are already differentiated into neuroblasts or functional neurones, that is to say, proliferation of cells probably does not occur in this group during the period under consideration.

8. The growing, terminal portion of the cord throughout the extent of the unsegmented mesoderm at least, in so far as it is differentiated from an epithelial condition, is purely sensory, and the sensory column of the region occupies the whole lateral portion of the cord.

9. Throughout the extent of the trunk the neurones have acquired their typical position and orientation in the cord and, at least in the medial region, have established a sensory tract of

fibers a considerable time before the embryo can respond to tactile stimulation.

10. The dorso-ventral distribution of the cells of the column is definitely correlated with the position of the mesoderm.

c. The giant ganglion cells as peripheral nerves

Certain results of my physiological experiments, which will be described in the physiological part of this paper, have required, for their explanation, a critical study of the peripheral relations of the giant ganglion cells, since the dendritic process of these cells constitute the afferent peripheral system of the trunk during the period under investigation.

For the illustration of these relations drawings have been made with the Bausch and Lomb drawing apparatus at a magnification of 940 diameters and reduced to 470 in figures 1 to 27 and 27a.

(1) *The relation of the giant ganglion cells to the skin.* The relations of these cells to the skin is illustrated particularly in figures 8, 15 to 21, 24 and 25.

Figure 8 is taken from a series of horizontal sections, but at this level of the cord the section is obliquely transverse with the dorsal portion pitched caudad. It represents a ganglion cell fiber in the space between the last two myotomes in a non-motile embryo. Here a large fiber, with a broad conical base coming out of the cord, at the latero-dorsal angle, reaches directly to the skin. Its area of attachment is upon the thickened region of the skin that characteristically projects in between the myotomes. There is evidence of its branching immediately beneath the skin and having at least two terminals.

Figures 15 to 17 are drawn from three successive sections of a transverse series. They represent a fiber in the myoseptum between the thirteenth and fourteenth myotomes of an embryo of the non-motile stage. The particularly significant relations here are between the fiber and the myotome as the fiber leaves the cord, the intimate contact with myotomes as the fiber passes between them and the pseudopodial branching of the fiber im-

mediately beneath the skin. Filaments from this region reach to the skin; others are directed towards the myotome.

Another direct connection of the giant ganglion cell with the skin in a non-motile embryo is illustrated in figure 18. This fiber occurs between the ninth and tenth myotomes. As in the case of figure 8, the fiber connects with the projection of the skin into the space between the myotomes.

Figures 19 to 21 are intended to show the intimate relation that holds between the skin and the spinal cord in the caudal region of the non-motile embryo, and the place that the giant ganglion cells hold in this region. These figures are taken from the level of the unsegmented mesoderm. In figure 19 the skin is in immediate contact with the spinal cord. In the region of the most intimate contact, where there is no perceptible boundary between the two structures, is a small mass of densely fibrillated substance. This, upon being traced caudad, is found to be continuous with the giant ganglion cell shown in figure 20. Dorsad of this region of most intimate attachment is another attachment of smaller extent and less intensive adhesion. Still more dorsad is an attachment which consists of filaments between the two structures. That there is here strong adhesion between the skin and spinal cord is evidenced by the rent that appears between the outer and inner layers of cells in the skin. There has been from some source a pull upon the outer layer of epithelial cells, tending to separate the skin from underlying structures, but the adhesion of the inner layer of epithelial cells to the spinal cord has proved stronger than that to the outer layer of epithelial cells. On the opposite side of the cord, also, in this section is the same evidence of adhesion and pull. In this side, however, the skin is partially pulled away from the spinal cord so as to tear out some of the fibrillar structures. These retain their connections in the cord at one end while they are attached to the skin at the other.

In the next section caudad from that of figure 19 the more dorsal and smaller of the adhesions in this figure gives place to fibrillar strands between the skin and spinal cord, and pigment

granules appear in the connecting filaments. Some of these granules appear to be suspended in distended portions of the filament-like particles in a slender pseudopodium. They are near the spinal cord and have the same characteristics as the pigment granules that appear in the peripheral portions of the cord. This relation suggests that these filaments are outgrowths of the cells of the spinal cord and that the larger adhesions may be secondary and not primary arrangements of the parts.

The giant ganglion cell of figure 20 has already been mentioned as sending its axone into the area of adhesion just described. From the basal portion of its process arises a small filament which is conical at its base and the internal structures of which merge into those of the cell. Other small filaments, apparently of epithelial origin, occur just caudad of this (the section is inclined from the dorsal side cephalad and ventrad). Farther cephalad is one of the characteristic connectives between the spinal cord and the skin. In this is a small yolk spherule. This inclusion has important bearing on the nature of the filament, for yolk is exclusively intracellular in its early embryonic relations in these animals. Therefore, since there are no mesenchymal cells in the vicinity to which the filament can belong, it must be either an integral part of a cell of the spinal cord or skin, or a syncytial connective between cells of the cord and skin. Further evidence of the cytoplasmic nature of these connectives will appear in the following paragraph.

Figure 21 illustrates a region of contact between the spinal cord and the skin in which the adhesion of the giant ganglion cell to the skin is accidentally shown. The lower part of the figure is directed caudad, so that the process of the giant ganglion cell (the most caudal of a group of three) must be regarded as an ascending process. Here again the rent between the outer and inner layers of the epithelia of the skin shows evidence of a pull having been exerted upon the outer surface of the skin. This probably occurred in the manipulation of the cut sections while they were being adjusted upon the slide. The adhesion of the fiber of the giant ganglion cell to the skin is shown by its relation

to the rent portions of the cord, it being pulled away from the outer parts of the cord along with the external limiting membrane. In this case also there are on both sides of the area of adhesion filamentous connectives between the skin and spinal cord, and in some of them yolk spherules are found. It is of particular interest here to note, further, that there are filamentous connectives across the rent of the skin, between the epithelial cells of one layer and those of the other. These connectives have the same structural and staining characteristics as have the connectives between the skin and spinal cord. If our interpretation of the cause of the rent in the tissue is correct, these connectives across the rent in the skin were fixed by the solution in their natural relations and afterwards torn from their normal setting in the epithelial cells. In other words, they may be regarded as intracellular structures that have been torn from their natural relations after fixation. In structure and staining reactions, then, the connectives between the spinal cord and skin have the appearance of cytoplasm.

Such adhesions as have been described between the skin and the spinal cord occur, during the period under investigation, only near the end of the tail bud, and particularly in the younger stages.

Figure 24 illustrates the manner of distribution of the fibers of the giant ganglion cells beneath the skin in the embryo of early-swimming stage. Mesenchyme cells are abundant in the vicinity of this fiber but its course is largely free from contact with them. Comparison of this with figures 8, 17 and 18 will give some idea of the changes that have taken place in the peripheral relations of these fibers in the transition from the non-motile to the early-flexure stage. In the later stage the individual fiber has wider extension or distribution than in the earlier condition, although in the latter there is connection with the skin on the part of these fibers throughout the extent of the segmented mesoderm; while in the more caudal region, as has just been shown, the relation between the giant ganglion cells and the skin in regions of adhesions is of such a nature as to admit of

stimulation of the cells of the sensory column through the skin. In figures 23 and 25, taken from farther cephalad in the embryos of the early-flexure stage, are shown the relations that such fibers as are illustrated in figure 24 assume with mesenchyme cells beneath the skin.

(2) *The relation of the giant ganglion cells to the myotomes.* Figures 1, 3, 4, 7, 10, 14 and 26 have been selected to illustrate the relation which the ganglion cells hold to the muscular system.

Figure 1 shows this relation in the embryo of the non-motile stage, at the level of the sixth myotome. This fiber projects from a giant ganglion cell through the dorso-lateral border of the cord and applies itself immediately to the caudal end of the myotome. Through the region of this contact it sends spinous processes in between the cells of the myotome. This is essentially the relation seen in figure 15, only from a different point of view. The relation of the fiber with the myotome is just as intimate as is the relation of any of the cutaneous fibers to the skin. The fiber is not simply passing across the surface of the myotome in close contact, but it has clearly differentiated processes that pierce the myotome. Similar relations are shown in figure 3. This contact also is with the end of the myotome. The peripheral fiber is here clearly seen to be a process from the same cell that sends its axone into the sensory tract.

In figure 4 appears an important bit of evidence on these fibers to the muscular system. Here at the level of the twelfth myotome, a fiber projects latero-dorsad till it emerges from the cord, then, instead of taking a short course through an open field to the skin, it swerves abruptly ventrad to the border of the myotome, upon which it spreads out in a disc-like terminal. It is difficult to conceive of any reason for a skin-sensory fiber to behave in this manner.

Figures 10 and 11 are taken from successive sections passing between the levels of the fifth and sixth myotomes cephalad of the unsegmented mesoderm in a non-motile embryo. Here the myotomes are closely pressed against the spinal cord, and the nerve fiber emerges from the cord at the dorsal border of the myotome. In both the sections the expansion of this fiber upon the

myotome and its projections in between the myotome cells are clearly shown. In figure 11 the relation of the terminal as it applies itself closely over a yolk spherule, partially encircling it, is typical of these endings. In this figure also the process from the same cell can be seen ascending into the sensory tract.

Figures 7, 12 and 13, taken from an embryo of the non-motile stage, illustrate the terminations of these fibers upon the muscles by smaller branches. In figure 7 there is a terminal upon one myotome and branches out towards the other; in figure 13, there are terminals upon both myotomes. Figure 12 is introduced because it represents one of the clearest cases of the relation of these finer branches of the nerve fibers to the myotomes. Within the cord, it should be noted, the process of the cell projects towards the myotome directly. Immediately after emerging through the external limiting membrane it divides into a more dorsal and a more ventral division. The destination of the dorsal division is not certain, although it apparently sends filaments into the myotome. The ventral division, however, sends a process directly against the myotome, where it spreads in both directions and pierces the myotome by fine, spinous processes. There can be no doubt about the origin and nature of this fiber and the only reasonable interpretation is that it, in part, terminates in the myotome as indicated in the figure.

Figure 14 is taken from the level between the second and third myotomes cephalad of the unsegmented mesoderm, in an embryo of the non-motile stage. It shows how the myotome at this level is closely pressed against the spinal cord so that a mere line marks the boundary between the structures. At the dorsal border of this contact a giant ganglion cell fiber emerges from the cord against the myotome at its end. As it passes across the end of the cells it sends the characteristic spinous processes into the myotome. Farther out it spreads out into an amoeboid film, which is applied to the myotome at one edge and extends out towards the skin at the other.

In figure 26 is shown the only case which has been observed where, by chance, the nerve fiber to the myotome has been disconnected, in part, from the myotome so as to show clearly the

nature of the terminals. The section from which this figure is taken passes between the sixth and seventh myotomes of an embryo of the coiled-reaction stage. The fiber here represented passes latero-dorsad from the border of the cord to a mesenchyme cell, where it branches and sends one division directly against the end of the myotome. At the end of the fiber it sends out claw-like processes into the myotome. But the most striking part of the structure is the series of spinous projections which beset the fiber through the last part of its course. These processes are clearly continuous with the substance of the fiber itself, and, by shifting the focal plane, one can clearly determine that they project outward from the fiber and upward in the preparation towards the observer. In this way they can be traced out into fibrillar structures that reach the limits of vision with ordinary oil immersion lens systems. To complete the picture, however, one must appreciate that the adjacent section of the series shows that the myotome, seen here only in contact with the end of the fiber, is shifted over the position of this fiber; so that these projections are clearly seen to be the terminal arrangement of the fiber upon the surface of the myotome. The spines along the border of the fiber are of the same sort as those at the end, but have been lifted away from their normal relation with the myotome. This preparation completes the picture of such conditions as are partially shown in figures 7, 10, 11, 12, 13 and 14.

The intimate contact between the spinal cord and the myotomes has already been mentioned. This relation is seen in its most complicated form in the caudal region of embryos of the non-motile stage, and is pictured in figure 22. This section is from a horizontal series but, with reference to the spinal cord in this region, it is directed obliquely from dorsad, cephalad and ventrad. The relation between the spinal cord and the myotome is here essentially the same as that figured between the skin and the cord in figure 19, only it is even more extensive. In many regions of this contact there is no perceptible border line between the cord and the myotome and the structures of one seem to pass over among those of the other and become indistinguishable from them. In the most dorsal portion of the area of adhesion is

found a giant ganglion cell which sends its process out into one of these fused region. In other words, this ganglion cell holds the same relation to the myotome as does that of figure 21 to the skin. Figures 21 and 22 are taken from the region of the unsegmented mesoderm of the same embryo.

In conclusion upon this phase of the work it may be confidently stated that the giant ganglion cells are muscle-sensory as well as skin-sensory in function. In fact the study as a whole gives me the impression that the muscle-sensory elements are more highly differentiated in the youngest stage studied than are the skin-sensory elements, but of course there is no means of determining this mathematically. In the youngest stage of this series both systems are established in their terminal relations with the peripheral organs.

(3) *The innervation of both skin and muscle by the same neurone.* Since the neurones of the sensory column innervate both muscular and cutaneous organs a question arises concerning the possibility of differentiating two sets of neurones in the system, the one innervating the skin and the other supplying the muscular system. The proposition of undertaking to make such an analysis of the system, however, is met with the unmistakable evidence that a single cell of this group, in many cases, terminates both in a myotome and in the skin. Several figures have been made to illustrate this relation.

Figure 2 is taken from the section adjacent to that of figure 1, and shows the continuation of the fiber, which has terminals in the myotome in figure 1, on out to the skin. Figure 6, in like manner, is from the section adjacent to that of figure 5. A composite of the two figures would give unmistakable evidence of one fiber branching and sending one process to the skin and the other to a myotome. In figure 9 this relation appears in one and the same section. Here the branching occurs near the skin and the branch that goes to the myotome has the characteristic ending described above in connection with specific treatment of the relations with the myotomes. Figure 8 is from the section adjacent to that of figure 9 and shows another branch of the same neurone terminating in the skin. The fiber of figures 15,

16 and 17, taken from successive sections also has the appearance of innervating the myotome at its base where it leaves the cord in figure 15, while it sends terminals to the skin in figure 17. In figure 23 there is a clear case of a large fiber from a giant ganglion cell sending a branch directly into the myotome, as it passes across the ends of the cells, and continuing on out to the skin. In figure 25 the giant ganglion cell fiber can be seen to pass through a groove in the end of the myotome and from this position to send a characteristic spinous process in among the cells of the myotome. The fiber in its further course branches out to the skin.

(4) *The number of peripheral fibers.* No special attempt has been made to count the number of peripheral fibers in the giant ganglion cell system, but some data on this point, extracted from my records of the general study, may be of interest.

The total number of fibers that were noted as adapted to special study and for written records was 186 in the thirteen embryos of the non-motile stage, and the largest number for any one specimen was 50. For seventeen embryos of the early-flexure stage the corresponding numbers are 185 and 57. In fourteen embryos of the coiled-reaction stage the total is 87 and the maximum in a single specimen, 28. For ten specimens of the swimming stage, the numbers are 115 and 36.

Further examination of my notes on the study of the giant ganglion cells and their fibers shows that of the 186 peripheral fibers studied specially in the non-motile embryos, and of the 185 studied specially in the embryos of the non-flexure stages, 136 in each case pass out over or in near relation to the ends of the myotomes. In the non-motile embryos, according to my judgment in studying from section to section 38 fibers were traced to the skin, 34 were regarded as terminating in the myotomes, and 20 had the appearance of ending both in the skin and in the myotomes. In the earlier embryos practically all the fibers observed had intimate relation to the ends of the myotome. In the older embryos occasional fibers, particularly in the more rostral regions, were observed to pass out directly to the skin over the middle part of myotomes.

Although the numbers tabulated above are not the results of a specific effort to count the fibers of this system, yet my impression, based upon my knowledge of the nature of all the material studied and the methods of study employed, is that it may be correctly inferred from them that there is no appreciable increase in the number of peripheral fibers in the transition from the non-motile to the early-flexure stage, that almost all the fibers in the younger stages pass out over or in close relation to the ends of the myotomes, and that, in the older stages, there are more fibers passing out to the skin over the middle portions of the myotomes.

In considering the numbers of fibers observed in the older embryos it must be kept in mind that there occurs during this period a rapid development of other structures that obscure the finer relations of the fibers. There results from this factor a much greater difficulty in following the fibers or detecting their presence and, to my mind, this explains why the maximum number of fibers recorded for any one individual of the two older stages is smaller than the maximum number found in the two younger stages.

(5) *Summary.* A summary of the peripheral relations of the giant ganglion cells may be outlined as follows:

1. The giant ganglion cells innervate both skin and muscle.
2. A single neurone of the system may innervate both skin and muscle.
3. These relations with the skin and myotomes become established throughout the extent of the segmented mesoderm some time before the embryo responds to tactile stimulation.
4. In the level of the unsegmented mesoderm the spinal cord and the giant ganglion cells have strong adhesions both with the skin and mesoderm, the substance of the cord in restricted regions becoming indistinguishable from the skin on the one hand and from the mesoderm on the other.
5. While there is no positive proof that there is an increase in the number of fibers to the skin during the period under investigation, there is clear evidence of a progressive differentiation and growth of the fibers, particularly in the extension and elaboration of the sub-epithelial plexus so that a single fiber acquires a

wider distribution, and in the association of the fibers with mesenchymal cells.

6. The great majority of the fibers of this system pass out of the cord into intimate relation with the ends of the myotomes. There may be in the later stages an increasing number that pass out to the skin over the middle portion of the myotomes or relatively free from them.

2. THE SPINAL GANGLIA AND THE LATERAL LINE ORGANS

That the anlagen of the spinal ganglia have no perceptible dorsal roots during this time has already been mentioned. Their condition in embryos of the early-swimming stage may be illustrated by the section through the fourth ganglion in figure 27. The cells here are closely crowded together into a compact group with definite border, but there are no clearly differentiated neurones among them. Comparison of the members of the series that appear in an embryo of this age shows that the anlagen of the ganglia which are situated in what may be termed the cervical and lumbar regions are larger than those in the mid-trunk region and farther caudad. This is obviously in anticipation of the sensory innervation of the limbs.

With view to a description of the lateral line system in a future paper the primordia of the lateral line organs of the head have been projected upon figures 56 to 59 along with the primordia of the organs in the trunk. Only a brief description of the distribution of these primordia in the trunk, however, will be given here, since a knowledge of their distribution and various experimental data have made it possible to eliminate the consideration of the lateral line organs from the fundamental physiological problems that constitute the real occasion for the anatomical part of the paper.

The appearance of the primordia as seen in section through the skin is represented in figure 27, which shows one of the structures at the level of the fifth or sixth myotome in an embryo of the early-swimming stage. The primordia of the youngest embryos of the series are not so clearly differentiated.

In the embryo of the non-motile stage no lateral line primordia occur caudad of the third myotome. Lying over the second myotome laterally, and extending partially over the first and third, is a broad primordium (fig. 56, *Po. LL. 2*), which lies immediately dorsad of an ectodermal thickening allied with the visceral system. In the embryo of the early-flexure stage (fig. 57) there are two such primordia, one (*Po. LL*) situated over the first myotome, and a long primordium (*LL*) extending over the second, third, fourth and fifth myotomes. The latter is constricted in its rostral portion, with apparently the tendency to separate into two primordia. In the embryo of the coiled-reaction stage the smaller primordium of the last stage seems to have coalesced with another which lay near the caudal border of the auditory vesicle, to form a single large primordium which extends from the auditory vesicle to near the second myotome. Farther caudad there are two primordia instead of one, as in the younger stage. The more rostral of the two (fig. 58) is short and lies over the third and fourth myotomes. The other extends through the level of the seventh, eighth, ninth and tenth myotomes.

As compared with the last embryo the oldest of the series (fig. 59) shows a considerable differentiation of the system of primordia. There is one large primordium over the first myotome, in addition to the one slightly cephalad and extending over the auditory vesicle. Ventrad of the second myoseptum is a small primordium (*Inf. LL*) which is situated just behind the external gills. It presumably represents the inferior line. In the position of the lateral line proper there are four distinct, short primordia scattered along over the eighth to twelfth myotomes, and a long one extending continuously through the thirteenth to seventeenth myotomes. In this embryo a dorsal group of primordia also appears, over the fourth, fifth, sixth and seventh myotomes, near the level of their dorsal border.

II. THE PHYSIOLOGICAL PART

In the treatment of the physiological part of this paper it is necessary, first of all, to show that the results of the various experiments which are to be discussed have to do with the afferent system of the trunk as opposed to that of the head, since the scope of this paper is explicitly limited to the problems of the sensory system of the trunk.

As already noted in the anatomical part, the definitive spinal nerves have no place in this problem since there are no dorsal spinal roots during the period under investigation. There is, however, more or less overlapping of the sensory field of the giant ganglion cells by the lateral line nerve and possibly by the cutaneous component of the vagus, and this community of area in distribution of the nerves from the two regions requires particular analysis. The anatomical details of this relation are left for a later consideration of the cranial system of nerves; but the distribution of the lateral line primordia has been given in the anatomical part of this paper, and the distribution of the general cutaneous component of the vagus has been described in my earlier studies on the cranial nerves of larval *Amblystoma* ('02).

Upon the basis of anatomical facts from these sources, one is able, by the simple experiment of transecting an embryo at about the level of the second myotome, to convince himself that the cranial nerves mentioned do not play any distinctive part in the reactions to stimulation upon the trunk, for the trunk of an embryo that has been transected in the manner indicated exhibits all the peculiarities of irritability that characterize the normal embryo. Indeed, there is not in all my experimental work upon the subject any evidence that the lateral line system during this period of development influences behavior in any way, or that there is any difference between the cranial nerves and giant ganglion cells as regards cutaneous irritability. There is no occasion, therefore, to question the validity of the experimental evidence to be presented in this paper concerning the receptive functions of the giant ganglion cells as the afferent system of the trunk.

1. THE REFLEX MECHANISM

In my earlier paper on *Diemyctylus* ('09) it was shown that trunk movements in response to stimulation upon the caudal portion of the trunk as well as to stimulation upon the head are initiated in the rostral portion of the musculature and that they progress from this region caudad as a wave of contraction through the myotomes of one side. While it is difficult or impossible to detect this characteristic of movement in amphibian embryos that develop rapidly and move quickly, the same cephalo-caudal progression of contraction clearly occurs in *Amblystoma* as has been described for *Diemyctylus*.

This feature of behavior is not only explained but made necessary by the fact that the motor innervation of the myotomes is by collaterals of neurones that constitute a continuous motor column in the spinal cord of the embryo, as shown in my paper on this subject ('13). My anatomical studies establish such a motor tract and column in the latero-ventral portion of the spinal cord. Numerous of my experiments corroborate these anatomical findings. By piercing the embryo, for instance, or by cutting it through from the ventral side so as to sever the ventral part of the cord while the dorsal portion is left intact, cephalo-caudal conduction, as evidenced by muscular contraction, has been intercepted without interfering with conduction caudo-cephalad past the lesion. In like manner, by inflicting a cut into the dorsal part of the cord, conduction caudo-cephalad has been intercepted without interference with the conduction cephalo-caudad past the lesion. In the former case, the muscle wave affects only the part cephalad of the lesion when the stimulus is caudad of the lesion; in the latter case, the entire trunk contracts in response to a stimulus that is applied cephalad of the lesion while no response at all can be elicited by stimulation caudad of the lesion. These experiments, although they do not establish the exact dorso-ventral extension of the tracts, certainly furnish ample physiological corroboration of my anatomical results, namely, that the most ventral part of the cord is motor and that the dorsal

portion is sensory. Furthermore, that the sensory and motor tracts of the same side are physiologically distinct and separate structures throughout the greater part of their extent is proved by the fact that the caudal piece of embryos that have been transected at certain levels, varying with the age, have no power of response to stimulation, either by tactile or chemical means. In fact, under normal conditions, the sensory and motor tracts of the same side seem to be absolutely isolated from one another physiologically, for reaction to a stimulus on one side of the embryo is typically followed by a contraction in the muscles of the opposite side, as my earlier work on *Diemyctylus* showed and as my later experiments on *Amblystoma* confirm. The explanation for the apparent exceptions to this rule in response is mentioned later on in connection with the proprioceptive functions of the giant ganglion cells.

The correlation of anatomical and physiological evidence, therefore, gives ample justification for interpreting the reflex mechanism of the trunk of these embryos as made up of a dorsal afferent system consisting of the giant ganglion cells, of a ventral motor system composed of a continuous, conducting column of neurones which innervate the muscles, and of an associative system, which, in the rostral portion of the cord, connects the afferent system of one side with the motor system of the other.

2. THE RECEPTIVE FUNCTIONS OF THE GIANT GANGLION CELLS

a. The interoceptive field

There is no evidence that the giant ganglion cells have an interoceptive field of stimulation or that the embryo of the age under consideration is influenced through any medium by its entodermal surfaces. The mouth is not formed till long after the period and the entodermal surfaces are not accessible to the typical stimuli of later life. The cloacal aperture of the archenteron is, of course established earlier, and it is conceivable that in the use of soluble substances for stimulating agents, these might diffuse into the cavity at a very slow rate and in exceedingly

minute quantities, but there is no suggestion of an influence from this source upon behavior, or of any nervous connection that could conduct stimulation from the field of the entoderm in the trunk to the spinal cord.

b. The exteroceptive field

The exteroceptive field of the giant ganglion cells must be considered with reference to tactile stimulation and chemical action.

(1) *Irritability to tactile stimuli.* From the later treatment of the action of soluble substances upon the skin it will be understood that tests for tactile irritability must be made with instruments that are chemically inert in water. As such a stimulating instrument, a hair, conveniently mounted in a holder, has been used in all my experiments, a method described in my earlier papers and adopted by Hooker ('11) to differentiate between tactile stimulation of the nerves and direct stimulation of the muscles.

The anatomical part of this paper shows that the cutaneous fibers from the giant ganglion cells are established in their typical relations with the skin in the non-motile embryo. Accordingly, it is found that when response begins, that is to say, in the early-flexure stage, irritability to tactile stimulation appears regularly over the trunk without any perceptible differentiation in cephalocaudal levels. In embryos of the early-flexure stage such subepithelial fibers as are shown in figure 24 are common, and, as is well known from the work of various authors which my own observations confirm, the fibers of some of the giant ganglion cells turn ventrad around the lateral border of the myotomes and pass far towards the ventral surface of the animal. Such structures as these account for a comparatively regular irritability over the entire surface of the trunk and tail bud, even to its tip. And as the dorsal and ventral fins expand into thin laminae these are irritable to their very margins.

In the very early-flexure stage response is irregular and uncertain when the stimulus is applied as a light touch to a single spot in the

skin, whereas the threshold of stimulation at such a time is much lower when the tip of the hair is moved gently over the surface of the skin. This is a conspicuous feature in the behavior of these embryos in the early period and is obviously to be accounted for upon the principle of summation of subminimal stimuli or alliance between reflexes. In the movement of the hair over the surface of the skin several giant ganglion cells, such as illustrated in figure 18, would be excited, or several endings of a single neurone such as that illustrated in figure 24. In case the stimulus excites more than one neurone, summation would seem to occur in the associative center; in case there is excitation only of several endings of a single neurone the summation phenomena could be referred only to the individual ganglion cell. It is reasonable to infer, however, that the movement of the hair upon the skin in the direction of the longitudinal axis of the embryo would stimulate numerous endings of several neurones, and that, therefore, there are exhibited here in this primitive reflex mechanism both the summation stimuli in the giant ganglion cells and the alliance between afferent stimuli in the associative center. There is nothing in this experiment upon which positively to base a differentiation between summation in the peripheral neurone and alliance of reflexes in the associative center; but since an experiment to be described later demonstrates conclusively the phenomena of antagonism between reflexes in these embryos there is strong presumption in favor of the idea that there is here effected an alliance between neurones that have been peripherally stimulated at intervals, for as the hair moves over the skin there is extension of both time and area of stimulation.

(2) *The action of hydrochloric acid as a stimulating agent.* Sheldon's work upon the reaction of the dogfish to chemical stimuli ('09) called my attention to the desirability of extending my studies on amphibian embryos to their reaction to substances in solution, with a view to correlating the action of chemical stimuli with definite structural elements in the nervous system. During several seasons, therefore, my attention has been given in part to experimentation upon this phase of the problem.

Preliminary experiments showed that response could be elicited by the use of the various substances to which the dogfish reacts in Sheldon's experiments, and that the action of the various inorganic acids is essentially the same so far as response is concerned. To simplify the experimentation, therefore, hydrochloric acid was adopted as a type of chemical stimuli. According to Sheldon's procedure a normal solution of the acid was prepared by titration against a gram-molecule solution of an alkali. From this stock of normal hydrochloric acid the various dilutions used as stimulating agents were made.

My first experiments consisted in spraying the solution against the embryo. As a means of applying such a stimulus with precision, pipettes were made from pieces of glass tubing, upon one end of which a very thin-walled bulb was blown while the other end was drawn out into a very fine capillary aperture. With the acid enough methylene blue was mixed to give it a perceptible color, so that the diffusion of the substance through the water could be followed under the microscope (the Zeiss binocular being used in these experiments). By this method a very fine jet of acid could be applied to restricted areas of the skin as comparatively localized stimuli.

It was necessary, of course, in the application of this method, to eliminate methylene blue and the mechanical impact of the spray as factors in stimulation. This was done by spraying the embryos with pure water and with a solution of methylene blue in pure water. Such check experiments showed that the mechanical impact of the spray and the methylene blue content of the solution were negligible factors so far as stimulation is concerned.

Embryos selected according to my typical physiological stages as determined by reactions to tactile stimulation were tested with various dilutions of the hydrochloric acid. Embryos of the non-motile stage, as determined by tactile stimulation, gave no response to the acid spray, while embryos that responded to tactile stimulation responded also to the acid stimulation when sprayed with dilutions as great as $n/400$. With stronger solutions the reactions were prompt and vigorous, while with greater dilutions

a latency period frequently followed stimulation. In embryos of the early-flexure stage this period was noted in some instances as being ten to twenty-five seconds in duration.

In embryos of the early-flexure stage reaction followed stimulation on the caudal portion of the trunk as well as on the head, and the movements were of the same nature as if they had been stimulated by tactile means. Furthermore, in case of transection of embryos it is found that caudal portions that respond to tactile stimulation respond also to chemical stimulation, and caudal portions that do not respond to tactile stimulation do not respond to chemical stimulation. In other words, in development and in mutilations response to chemical stimulation comes and goes hand in hand with response to tactile stimulation, and the two forms of stimuli excite the same forms of response. These facts create a strong presumption against the idea that there are different mechanisms involved in the reactions to the different forms of stimulation.

By the use of a spray it is obviously impossible to determine the threshold of stimulation exactly, because of the increasing dilution of the acid as it diffuses through the water. To study this point more carefully and study comparatively the action of different concentrations of the acid, embryos were immersed in various solutions and their activity observed. The following experiment, which has been repeated with various modifications, may be accepted as representative of the results of this method of study.

Four *Amblystoma* embryos of the early-swimming stage were immersed in similar dishes containing the following solutions: (a) cistern water, from the stock used for growing the embryos in the laboratory; (b) distilled water which had been aerated by pouring from one dish to another repeatedly; (c) HCl n/2000; (d) HCl n/3000; (e) HCl n/4000. The distilled water used in diluting the normal solution of the acid had also been aerated in the manner mentioned.

At the expiration of five minutes the greatest activity was shown by the embryos in the dish with HCl n/2000. Two minutes later there was slight activity among the embryos in HCl n/4000,

although during the first ten minutes of the experiment the embryos in distilled water manifested as much disturbance as did those in HCl n/4000, and at times apparently more. Thirty minutes after the beginning of the experiment the embryos that were immersed in HCl n/3000 showed the greatest activity; those in distilled water were slightly active; those in HCl n/4000 were perfectly quiet. It was observed throughout the experiment that the movements of the embryos in the acid solutions were of a more convulsive nature than were the movements of those in distilled water. Two hours and five minutes after the beginning of the experiment the embryos in HCl n/3000 were still the most active; those in HCl n/2000 were totally inactive; those in HCl n/4000 and in distilled water were slightly active. During the whole experiment up to this point no movements had been observed among the embryos in the cistern water. At the expiration of four hours and twenty-five minutes after the immersion in the solutions, the specimens in HCl n/3000 and those in HCl n/4000 were more active than those in distilled water. An hour later the embryos in HCl n/4000 were still more active than those in distilled water.

During the progress of this experiment it became obvious that at least not all the reactions that were occurring in the various acid solutions could be regarded as due to a normal process of stimulation, for within three hours after the beginning of the experiment the four specimens that were immersed in HCl n/2000 showed marked shriveling of the margins of the fins and the skin had become pale. Upon the discovery of this destructive action, these four specimens were removed and two others introduced into the identical solution. These became completely inert to ordinary means of tactile stimulation within forty-five minutes after immersion in the solution. Further observations showed that the specimens that were immersed in HCl n/3000 for four hours had undergone various degrees of decline in irritability to tactile stimulation, and that one had wholly lost the power of response to such stimulation. Prolonged immersion in HCl n/4000 also proved to have a very perceptible injurious effect upon the skin of the embryos.

As a result of experiments along the line indicated above, the leading question in my experimentation with acid took this form: can a concentration of acid be found that will stimulate and not destroy the skin; and, if so, is there anything in the nature of the response that differentiates normal stimulation from destructive actions by substances in solution?

As a means of studying this aspect of the question the method of recording movements with a myograph was devised, and used according to the method described in connection with figure 60. Studies were made with this method upon the action of hydrochloric acid in as great dilution as $n/10,000$; with the result that degrees of concentration of the acid which are not adequate to stimulate the reflex mechanism were found to have a destructive action upon the skin perceptible under the microscope.

In figure 60, graphs *A*, *B* and *C* represent respectively the action of HCl $n/300$, $n/400$ and $n/500$ upon the behavior of embryos of *Rana catesbiana* of the advanced swimming stage. The solid line of each graph represents the composite of the activity of five specimens in the acid, while the broken line represents the composite of the activity of the same five specimens in pond water following the same kind of mechanical agitation in changing from dish to dish as occurred in the manipulation with acid. The reaction in water was in each case taken immediately before the reaction in acid.

In the three graphs on the action of acid there is a striking similarity; but the most noteworthy result of this method of study is that the composite of the activity of a number of specimens can be represented by a curve. Particularly is this impressive when one knows that, although these very embryos were removed from the acid immediately following the experiment (after an immersion of less than seventy-five seconds) they showed unmistakable evidence of the destructive action of the stimulating agent upon the skin. In addition, these graphs seem to show, particularly when the increasing normal activity as indicated by the broken line is taken into account, that the intensity of the response (the height of the curve) increases, while the duration of the response (the length of the curve) decreases

with the degree of concentration of the acid, that is to say, the intensity of the response tends to vary directly with the destructive action of the stimulating agent while the duration of the response tends to vary inversely with the destructive action.

Myograms made by this method on certain embryos under the action of HCl n/1000 showed no positive evidence of stimulation; yet immersion in the same solution for a period of fifteen minutes caused pronounced disintegration of the skin. Prolonged study with these quantitative methods has given a mass of unquestionable evidence in favor of the conclusion that the action of hydrochloric acid upon the skin of amphibian embryos cannot be regarded as a normal stimulation of nerve endings or sensory cells of any sort.

Various observations by other methods have confirmed this conclusion. If, for instance, as observed also independently by a student in my laboratory, Mr. M. W. Shipley, an embryo of *Amblystoma* is immersed in a dilution of HCl which does not excite movement, and is, after a brief period, transferred again to the original medium of pond water, it is, by this last immersion, excited to a long series of convulsive movements. This characteristic of the action of HCl on the skin was studied closely, in one case, upon 40 embryos of *Amblystoma* in the coiled-reaction stage. In this experiment each embryo was first observed for one minute in a dish of pond water and its movements noted. It was then placed in HCl n/1000 and observed for the same length of time. Immediately following this it was replaced in the pond water from which it was originally taken. Through the whole process every movement was recorded. As a result of this experiment upon forty embryos the following conclusions were drawn: (1) There was no great variation among the embryos in the degree of normal activity in pond water. (2) There was great variation among the embryos in irritability to the acid. (3) There was great variation among the individuals in their irritability to pond water after the bath in HCl, and there is a distinct correlation between irritability to the acid and the subsequent irritability to pond water. (4) There was, on the whole, much greater activity in the pond water following the bath in

acid than there was in the acid. According to the records of the experiment, there were five movements among forty embryos during the minute in the pond water originally, indicating the degree of normal activity. In the HCl $n/1000$ there were 60 movements during the same period, while there were 151 movements during the one minute of immersion in pond water after the bath in HCl $n/1000$. These data certainly prove that, while the acid excited response, it rendered the skin abnormal in some respect.

With view to determining the nature of the action of very dilute solutions of hydrochloric acid upon the skin of these embryos, specimens were selected which had not nearly reached the stage of earliest response and which exhibited the typical ciliary movement over the surface of the skin. In these embryos the reaction of the skin alone was studied without the intervention of nervous or muscular phenomena.

Embryos of this age, when immersed in pure water in which fine granulated carmine is suspended, keep the surface of the body clear of this substance indefinitely. In such a preparation the carmine particles may be seen under the microscope in a perpetual stream over the surface flowing cephalo-caudad and off at the caudad end of the animal. If, however, the embryo is immersed in HCl $n/1000$ in which finely pulverized carmine is suspended, the particles begin to adhere to the surface of the skin in less than two minutes. The cilia beat vigorously among the accumulating carmine particles but fail to dislodge them so long as the embryo remains in acid. Immersion in a stronger solution of the acid produces this effect more quickly and in a short time causes the exudation of globules of adhesive substance on the surface of the ectodermal cells. Prolonged immersion intensifies this action till the complete disruption of the cells occurs. And when this disruption occurs it is most pronounced in the regions where the particles of carmine first adhere in the dilute acid solution. Parker ('12) places the threshold of stimulation by hydrochloric acid in the mouth of man at $n/1000$. This being correct, the ectodermal cell of the amphibian embryo which has no nerve supply responds directly to as great a dilution of the acid as does the

highly differentiated and innervated surfaces in the oral cavity of man. But this is not all, a distinctly perceptible reaction of the ectoderm cells of these embryos has been observed in HCl n/10,000.

From these and from numerous other experiments with hydrochloric acid as a stimulating agent, the following conclusions have been drawn:

1. The ciliated cutaneous epithelial cells of young *Amblystoma* embryos react directly to exceedingly dilute solution of hydrochloric acid without the intervention of a nervous system or nervous connection of any kind.

2. No degree of concentration of the acid can be found which will excite muscular response without causing destructive processes in the skin.

3. There is a distinct correlation between the nature of the muscular response and the injurious action of the acid which is used as a stimulating agent.

4. There is no evidence of a normal irritability of the skin to acid.

5. If there should prove to be such a normal irritability, it must necessarily act upon the same reflex mechanism as that through which reaction to tactile stimulation takes place.

c. The proprioceptive field

The anatomical part of this paper has dealt in detail with the endings of the giant ganglion cells upon the myotomes. That such endings are in fact sensory is demonstrable experimentally.

It has been explained in various connections that, during a certain period of development, particularly during that period which is characterized by the coiled-reaction, these embryos move almost constantly away from the side touched. When the embryo reaches the swimming stage, however, the movements become more irregular in direction relative to the side stimulated, and with further development every suggestion of this law of response that prevailed in the earlier period disappears. If, however, an embryo of the advanced swimming stage, which has

lost the characteristic crossed reflex, be immersed in a solution of curare, the extent of the movement in response to tactile stimulation gradually becomes reduced, and the forms of movement go out of the behavior in the reverse order to that according to which they appeared, that is to say, the effective swimming movement gives place to feeble S-reactions; these, to coiled-reactions; these, to feeble flexures; till eventually only the slightest head movement occurs, and finally responses of all kinds cease. Now, during the later period of the decline in motility, when behavior has reverted to the earlier type, the regularity of crossed response reappears, and through long series of responses the movements will be constantly away from the side stimulated. The most obvious inference to be drawn from this recurrence in the form of behavior is that the progressive paralysis of the motor nerve endings by curare eliminates more and more the stimulations of the muscle sensory endings of the giant ganglion cells, and the associative center or motor column, one or both, are left to the almost exclusive stimulation from localized cutaneous areas.

These results from experiments with curare are confirmed by another simple form of experiment, namely, the transection of swimming embryos at about the level of the pectoral limb bud, so as to leave in the head piece just enough muscle to give a perceptible contraction when observed under the microscope. The head piece of such a transected embryo, it is found, through long series of reactions approaching a hundred in rapid succession, contracts the muscles constantly on the side opposite the stimulus. Here, again, the associative and motor centers have been severed from the influence of the greater part of the trunk, and the dominating factor of the trunk as compared with the cranial field of stimulation is the muscle system.

From such experiments as these, it seems necessary to conclude that the giant ganglion cells have a proprioceptive field of stimulation through their endings on the myotomes, and that this field of stimulation has a profound influence over the behavior of the animal when it responds to localized cutaneous stimulation. This primitive reflex mechanism, therefore, exhibits the phenomena of

antagonism between reflexes after the manner of the mammalian reflex arc.

In my paper upon *Diemyctylus* ('09) attention was called to the secondary movements which occur in the behavior of these embryos, that is to say, movements that frequently occur immediately after the initial response or before it is completed. These movements might be accounted for upon the hypothesis that the motor or associative cells act rhythmically to a single stimulus, only for the fact that the secondary movement sometimes exceeds the initial movement in extent. Although there may be a tendency for these cells to act in rhythm, there must be something besides the rhythmic activity to account for the acceleration of movement. In the light of the anatomical and physiological demonstration of a proprioceptive field of stimulation it is more reasonable to suppose that stimuli from this field, through imperfect alliance with stimuli from the cutaneous field, arouse the secondary movements. We may recognize in these movements, therefore, the phenomena of alliance between reflexes. The movements, in and of themselves, have the characteristics of the reflex after-discharge of the mammalian reflex arc, and, indeed, regardless of the above interpretation of their immediate cause, they may be perfectly analogous to the reflex after-discharge, since the latter response of the mammalian reflex arc may involve both the rhythmic action of neurones and excitation from the proprioceptive field.

The anatomical part of the paper brought out the fact that a single giant ganglion cell may innervate both skin and muscle. Some of these cells are apparently distributed exclusively to the skin; others may be distributed exclusively to the muscles; but some certainly go both to skin and muscle. In the consideration of the interaction of stimuli from the skin on the one hand and from the muscles on the other, it is necessary, therefore, to assume that there is no physiological differentiation between the stimuli from the different sources. Exteroceptive and proprioceptive stimuli might become allied, or the one reinforce the other, within one and the same neurone.

These facts lead further to the conclusion that the impulses from the proprioceptive field follow the same conduction paths as do those which originate in the skin. This means that action in the muscular system of one side tends to excite contraction in the muscles of the opposite side. This relation, indeed, may be the paramount factor in adaptation that determined the integration of the nervous system into physiologically distinct longitudinal paths on the same side and crossed paths only in the cephalic portion between the afferent path of one side and the efferent path of the other, for through such a mechanism one act of the swimming movement would stimulate the next with the result of serial contractions which effect locomotion. The question as to whether proprioceptive or exteroceptive stimulation was the primary concern of the giant ganglion cells resolves itself, of course, into pure conjecture; but in ontogenesis these two functions are obviously merged into a common action through one and the same mechanism.

III. DISCUSSION OF RESULTS

The literature upon the subject of the giant ganglion cells is very extensive and no effort will be made here to review it in detail. Comparatively recent critiques upon the literature have been offered by Dahlgren ('97), Van Gehuchten ('97), Harrison ('01) and others, and since the contributions on the subject are almost exclusively morphological with little or no reference to the related physiological problems, and since the investigations have been made upon the widely divergent forms with more or less disagreement among the morphologists, it is only in the way of corroboration of my own anatomical findings that any help has been drawn from the literature upon my specific problem of correlation of function and structure in the particular animals in hand. My investigations were not undertaken with the idea of discovering new things in anatomy; they were undertaken with a view to correlating specific structure, in particular animals, with known physiological characteristics of those animals. My physiological experiments therefore, and not the observation of others, have been my guide and my check.

As might be expected, my observations add little to what has been well known concerning the form and orientation of the giant ganglion cells within the spinal cord. These features of the cell have been exhaustively treated by Beard ('92, '96), Dahlgren ('97), Studnička ('95), Tagliani ('95), Sargent ('98), Johnston ('00), Harrison ('01) and others. Some authors, including Johnston, regard the cell process that is directed caudad in the cord as a neurite, and therefore interpret the column as physiologically descending as well as ascending. This may be true in the animals studied by these investigators but there is no evidence of descending impulses in the column of the giant ganglion cells in *Amblystoma* embryos used in my work, and, in some cases, the peripheral fiber has been observed to arise from the descending process of the cell.

The lateral position of the cells in the more rostral portion of the cord and their occurrence well forward in the medulla have been observed by Johnston in *Catostomus*. He observes also that in *Catostomus* and *Coregonus* the nuclei are differentiated from the surrounding nuclei in their staining reaction, being colored red while the surrounding nuclei are colored green with the Ehrlich-Biondi triple stain. With the use of orange G, Lyons blue and erythrosin as cytoplasmic stains my preparations show the nuclei of the giant ganglion cells tinged slightly with these colors, in this manner differentiated from the nuclei about them.

My results add to the knowledge of these cells as regards their central relations chiefly in demonstrating their organization into an afferent conduction path which, through the greater part of its extent if not through it all, is physiologically distinct from the motor tract of the same side. My findings show that this column of cells is an integral part of a reflex mechanism which has the essential characteristic of the typical reflex arc of higher vertebrates.

Upon the peripheral relations of the giant ganglion cells there has been some difference of opinion. Studnička ('95) regards the peripheral processes as motor upon the basis of his observation that they terminate in the myotomes in *Rana* and *Bufo*. Tag-

liani and Beard (in his earlier paper) are quoted by other authors as being of the same opinion with Studnička. More recent observers, however, discredit this interpretation and fail to find the endings of the fibers in the myotomes. The general interpretation, which has been insisted upon in recent years, that the dorsal part of the cord is sensory, probably created a presumption in the minds of later observers against the idea that these fibers end in the myotomes, for such endings had been interpreted as motor. My anatomical preparations, however, seem to be unequivocal on this point, and my physiological results are equally positive concerning the existence of a functional proprioceptive field of stimulation. The harmony between my anatomical and physiological observations adds weight to my conclusion that the giant ganglion cells are muscle sensory as well as skin sensory in function.

That the same neurone may carry impulses from the skin and the muscle as my anatomical findings indicate is also against the presumption of current anatomy and physiology. In the consideration of this point, however, it should be borne in mind that the giant ganglion cell is obviously a primitive structure. This seems to be conceded by all morphologists who have studied the subject. In my opinion, the giant ganglion cells represent the afferent element of the nervous mechanism of the earliest chordates that propelled themselves in locomotion by means of a mesodermal muscular system. In such an ancestral form, as in the ontogenetic stage of development now under consideration, physiological differentiation in the synaptic centers between cutaneous and muscle sensory impulses could be of little, if any, significance. The paramount feature of adaptation of the reflex mechanism of these embryos is immediately centered in locomotion and not in differential sensory functions. From the point of view of the efficiency of this mechanism as it actually works in the life of amphibian embryos there is no reason apparent why impulses should not be carried from the skin and the muscle to the spinal cord through the same neurone.

With reference to the subepithelial structures of the giant ganglion cells, the repetition of Wintrebert's ('04) experiments

by Hooker ('11) is of interest. Hooker's experiments were made to test the property of the skin as a conductor of impulses longitudinally in the trunk. Incidentally they lend credence to the idea, if they do not certainly demonstrate, that the subepithelial terminals of the giant ganglion cells do not form a conducting syncytium in or beneath the skin. This is in harmony with my experiments in which severing of the dorsal portion of the cord made it impossible to stimulate response to a light touch applied caudad of the lesion. There is obviously here no cutaneous or subcutaneous structure which can conduct stimuli longitudinally in the trunk for any considerable distance.

My experience with hydrochloric acid as a stimulating agent have important bearings upon the work of Parker ('12), Sheldon ('09), Cole ('10) and others upon fishes and Amphibia with reference to a general or "common chemical sense" in the skin. These authors hold the view that there is a special set of receptors which are normally irritable to various substances in solution. My conclusions do not harmonize with this view so far as amphibian embryos are concerned. Moreover, a comparison of my results in detail with those of Parker, Sheldon and Cole convinces me that the burden of proof is still upon them as regards the nervous irritability of the skin to chemical stimuli in fishes and amphibians generally; for every essential characteristic of response which they describe can be duplicated in amphibian embryos which are known to be responding to a violent disruptive action of the stimulating chemical agent upon the epithelium of the skin.

To understand how the response of fishes and amphibians to acid, or other substances in solution, may be caused by the destructive action of the stimulating agent, it is only necessary to recall that the deeper cells of the cutaneous epithelium of all vertebrates are permanently embryonic; and that the general cutaneous nerve fibers end among these embryonic cells as the terminals of giant ganglion cells end upon the deeper layer of epithelial cells of the amphibian embryo. My experiments upon young, ciliated embryos with HCl $n/1000$ and $n/10,000$ show that the cutaneous epithelial cells of the embryo, regardless of nerve endings, are beyond comparison with the skin of fishes in sen-

sitiveness to the action of acid. They would, in fact, explode almost instantaneously upon immersion in such concentrations of acid as were used by Parker and Sheldon in their experiments upon fishes. It is my opinion that the deeper embryonic cells of the skin of adult fishes and amphibians would act in the same way if exposed directly to the acid. In their normal condition, however, they are bound down and protected by a thick, less sensitive and more impervious layer of cells. Under such condition the acid must cause exceedingly violent mechanical disturbances beneath a comparatively passive exterior; while the less sensitive outer layer of cells protect the deeper cells during processes of repair when the destructive action ceases.

Sheldon's experiments in which he found that areas of the skin which have become fatigued to tactile stimulation are still sensitive to various chemical agents, and that areas that have been stimulated by chemical agents are thereafter for a time insensitive to tactile stimulation, are exactly in line with my observation of embryos immersed in HCl $n/2000$, $n/3000$ and $n/4000$. His experiments mean to me that the chemical agent has not fatigued but destroyed the sensitive portions of the skin to such an extent as to render it inert to normal stimulation; while the "fatigue" to tactile stimulation is merely adaptation, just as my ear is now so adapted to the sound of the clock that the tick does not affect my motor system. These experiments of Sheldon's, to my mind, illustrate exactly the difference between the effect of normal stimulation and destructive action by stimulating agents.

In like manner, Cole's experiments with cocaine upon frogs which were stimulated with chlorides are not conclusive on the point for which the author is contending. In interpreting this experiment it must be considered that the action of the chloride is much more extensive and affects many more cells at once than does any of the single acts of pinching or pricking which Cole employed to test the effect of the cocainization upon the receptors to tactile stimuli. Furthermore chemical action has a much more violent and destructive effect upon protoplasm than does mechanical stress and strain, as may be experienced, for instance,

in the manipulation of growth cultures of microorganisms. In short, in testing the effect of cocaine upon the irritability to the chloride, Cole applied stimuli which in both extent and intensity are beyond comparison with the stimuli which he used to test the effect of cocaine upon the irritability to tactile stimuli. Furthermore, Cole's observation that abrasions in the skin increase the irritability to acid, or shorten the reaction time, is also in harmony with the hypothesis that the action of the chloride is destructive and not a normal physiological stimulus.

When the epithelial cell itself, without the intervention of any nervous structure, is sensitive to as high a dilution of hydrochloric acid as are the taste organs of man (Parker ('12) estimates this at $n/1000$), it is not surprising that the oral surfaces of the shark should be sensitive to $n/75$ (Sheldon); nor does a special set of receptors seem necessary to enable *Ammocoetes* to respond to $n/40$ (Parker); or *Ameiurus*, to $n/2$ (Parker) when applied to the skin of the trunk. Such concentrations of acids as this are beyond comparison with the degree of concentration which will act destructively upon epithelial cells. Such strengths of chemical stimulation certainly have no place in the normal environment of the animals upon which they were used experimentally, and the idea that there is a special set of receptors, or nerves (Sheldon), to take account of such stimuli even in these animals should have absolutely unequivocal and uncontradictory evidence in its favor before it is adopted as a general biological principle.

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ABBREVIATIONS

<i>Ad.</i> , areas of adhesion between spinal cord and skin or mesoderm	<i>Mes.</i> , mesenchyme
<i>APDC.</i> , ascending process of the giant ganglion cell	<i>Ms.</i> , muscle-sensory ending of giant ganglion cell
<i>Aud. V.</i> , auditory vesicle	<i>Opt. St.</i> , optic stalk, cut at the surface of the brain
<i>C.</i> , notochord	<i>Po. LL.</i> , postauditory lateral line primordium
<i>CC.</i> , central canal of the spinal cord	<i>Pr. LL.</i> , preauditory lateral line primordium
<i>Com.</i> , commissural cell	<i>R. V, VII, etc.</i> , positions of the roots of the corresponding cranial nerves
<i>DC.</i> , giant ganglion cell	<i>S.</i> , spinal cord
<i>DCF.</i> , peripheral fiber from the giant ganglion cell	<i>SG.</i> , anlage of the spinal ganglion
<i>Dor. LL.</i> , primordium of the dorsal division of the lateral line	<i>Sub. LL.</i> , suborbital lateral line primordium
<i>DT.</i> , sensory, or dorso-lateral tract, arising from giant ganglion cells	<i>Sup. LL.</i> , supraorbital lateral line primordium
<i>Ec. Th.</i> , ectodermal thickenings connected with the visceral pouches	<i>VC.</i> , motor cells, the cells of origin of the motor, or ventro-lateral tract
<i>Inf. LL.</i> , primordium of the inferior or ventral division of the lateral line	<i>VT.</i> , motor, or ventro-lateral tract
<i>LL.</i> , primordium of the lateral line	<i>Y.</i> , yolk spherule
<i>M.</i> , myotome	<i>Z.</i> , Cytoplasmic connectives between the spinal cord and the skin.
<i>Md. LL.</i> , primordium of the mandibular line	

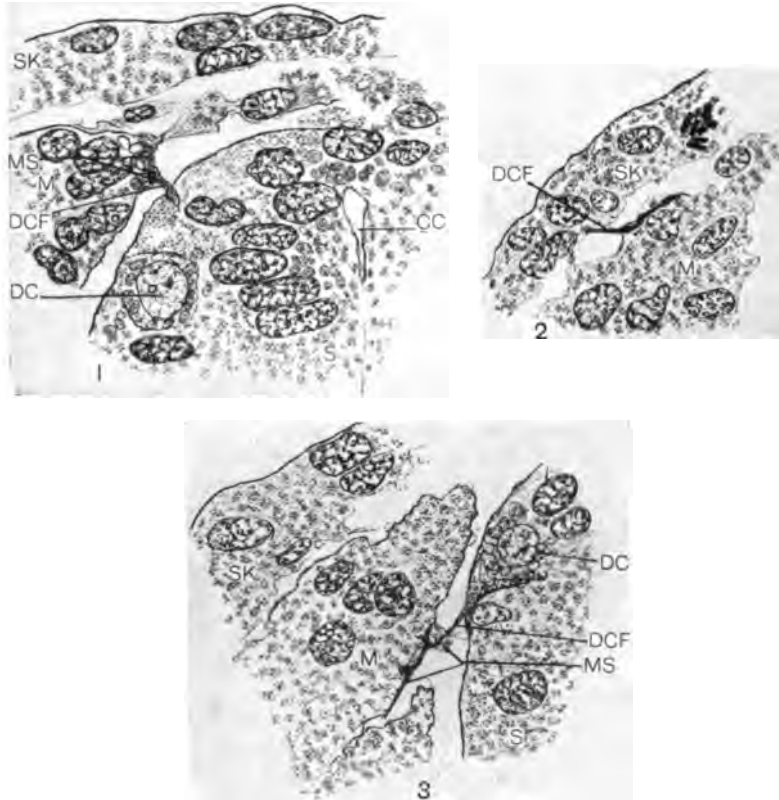


Fig. 1 From an embryo of non-motile stage, level of sixth myotome. Fixation in corrosive acetic mixture; stained with Boemer's hematoxylin and acidulated orange G; transverse plane; 10μ . Figures 1 to 27 are all magnified 470 diameters.

Fig. 2 From the same specimen, level of sixth myotome.

Fig. 3 Same specimen, level between eighth and ninth myotomes.

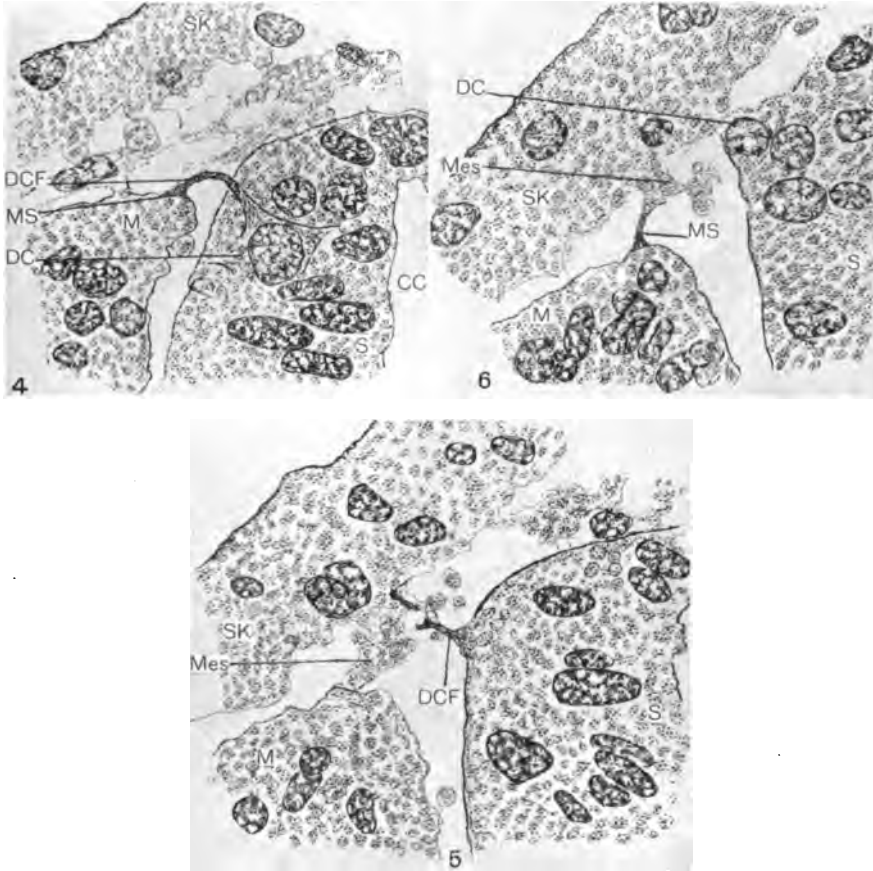


Fig. 4 Same specimen, level of the twelfth myotome.

Fig. 5 Same specimen, level of caudal end of fifteenth myotome.

Fig. 6 Same specimen, level of caudal end of fifteenth myotome.

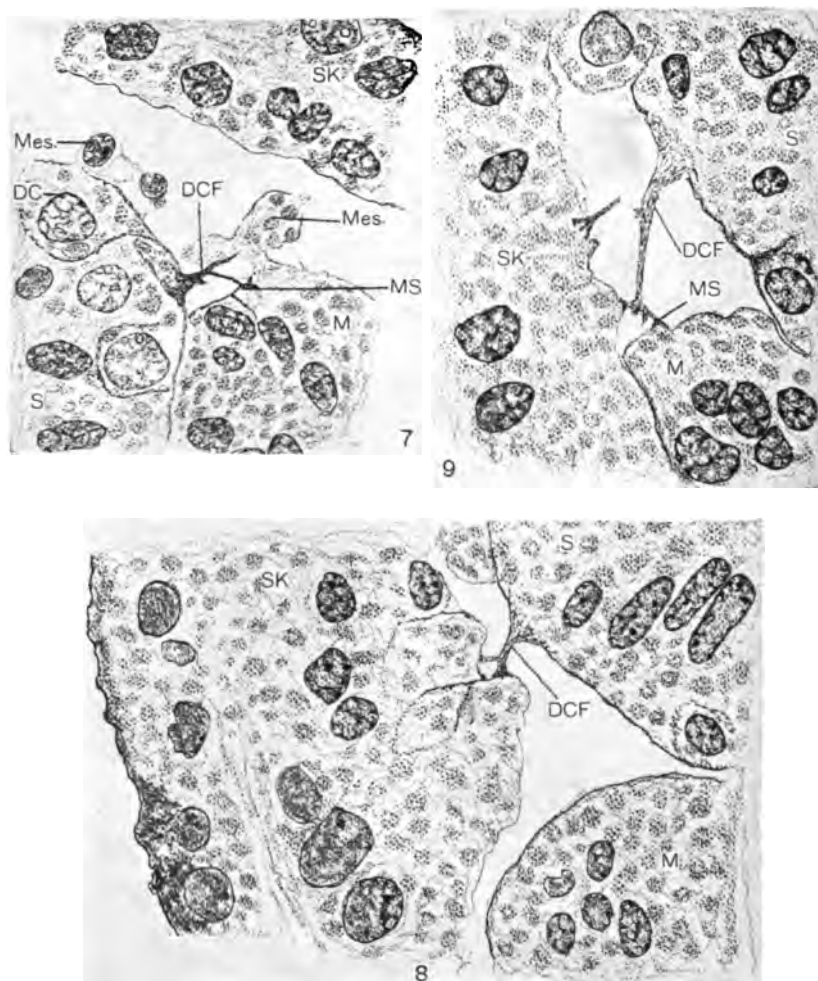


Fig. 7 From an embryo of the non-motile stage, between the eighteenth and nineteenth myotomes. Fixation, Van Gehuchten's fluid (alcohol, chloroform, acetic acid); stained with erythrosin and toluidin blue; horizontal plane, 5 μ .

Fig. 8. From an embryo of the non-motile stage, between the last two myotomes cephalad of the unsegmented mesoderm; fixation and staining like the last; transverse plane, 10 μ .

Fig. 9 Same specimen, from section adjacent to the last.

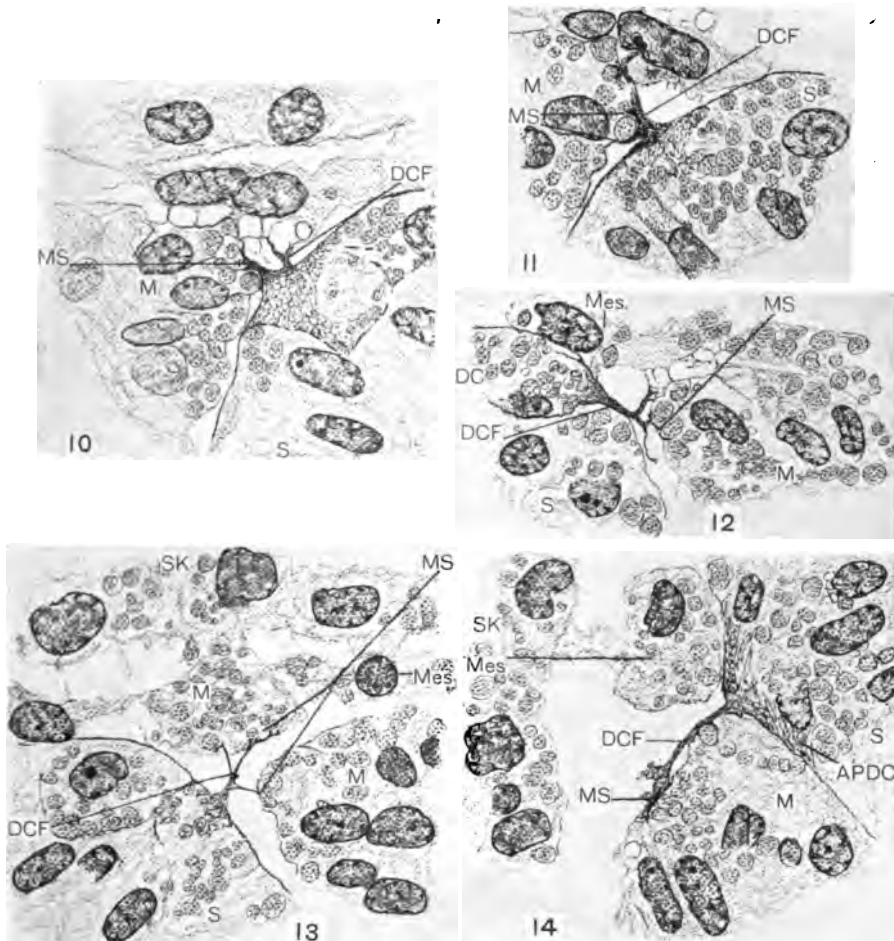


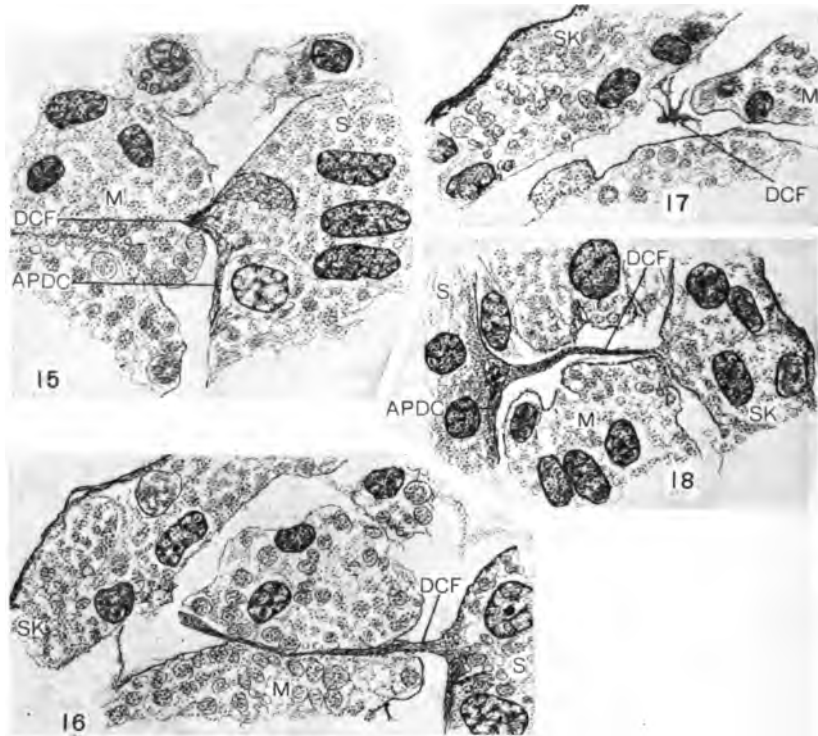
Fig. 10 From an embryo of the same age, same fixation and same staining as the last; transverse plane; 10 μ . At the level of the fifth and sixth myotomes cephalad of the unsegmented mesoderm.

Fig. 11 From a section adjacent to the last.

Fig. 12 From the same embryo; two sections removed from the last.

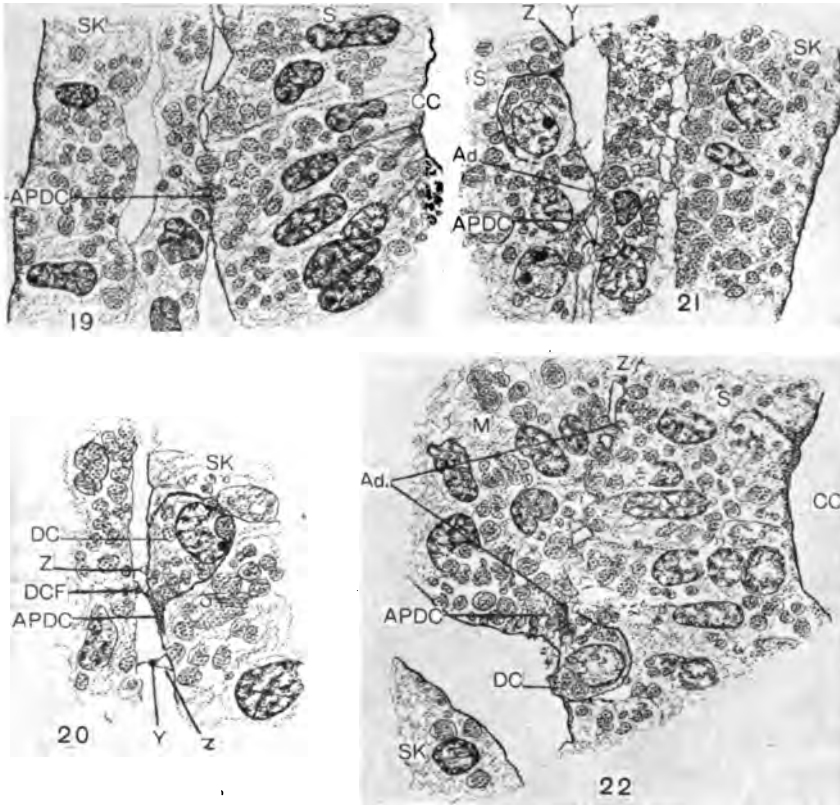
Fig. 13 From the same section as that of figure 11.

Fig. 14 From the same embryo, between the second and third myotomes cephalad of the unsegmented mesoderm.



Figs. 15, 16, 17 From an embryo of the non-motile stage, between the thirteenth and fourteenth myotomes. Same fixation and staining as figure 7. Transverse plane; 5 μ .

Fig. 18 From a non-motile embryo, at the level between the ninth and tenth myotomes. Fixation in formalin-Zenker; Delafield's hematoxylin and acidulated orange G; horizontal plane; 10 μ .



Figs. 19 to 22. From the same embryo as figure 7, in the level of the unsegmented mesoderm.

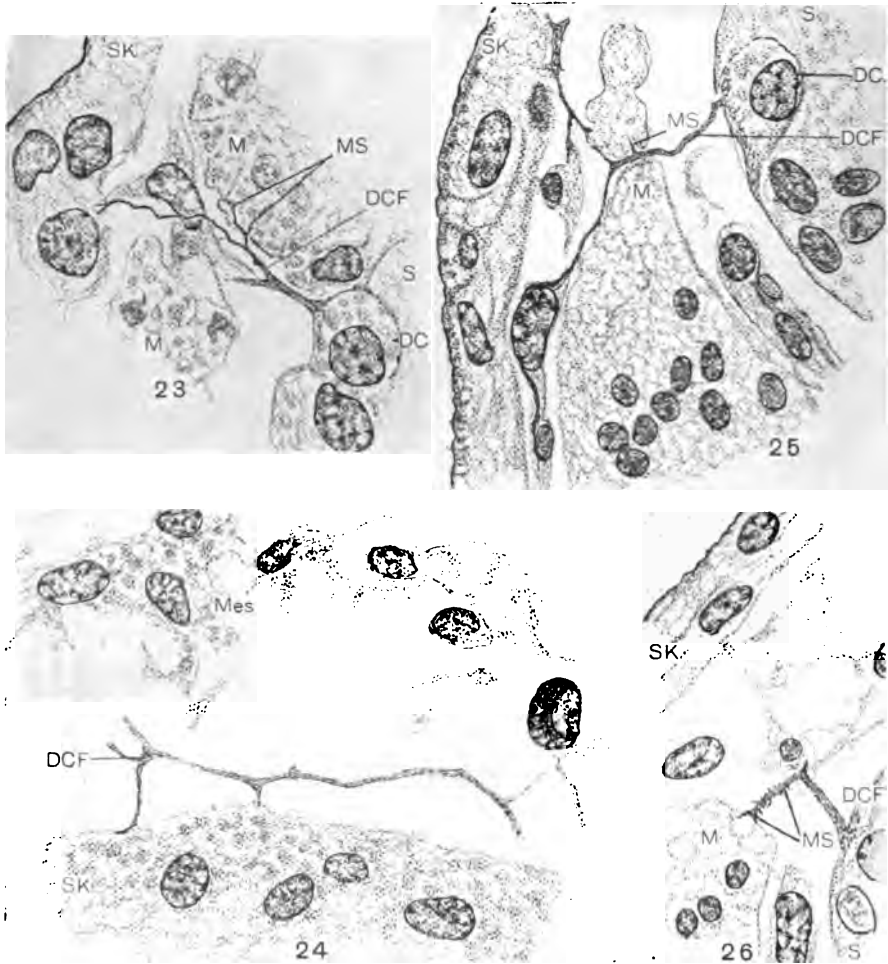


Fig. 23 From an embryo of the early-flexure stage, in the rostral portion of the trunk. Fixation in corrosive sublimate-acetic acid mixture; stained in alum carmine and Lyon's blue; horizontal plane; 10 μ .

Fig. 24 From an embryo of the early-flexure stage, at the level of the eighteenth myotome. Same fixation and staining as figure 7; horizontal plane; 7 μ .

Fig. 25 From an embryo of the coil reaction stage, at the level between the fourth and fifth myotomes. Fixation in corrosive sublimate-acetic acid mixture; stained in alum carmine and Lyon's blue; transverse plane; 10 μ .

Fig. 26 From the same specimen, at the level between the sixth and seventh myotomes.



Fig. 27 From an embryo of the early-swimming stage, at the level between the fifth and sixth myotomes. Same treatment, plane and thickness as in case of figure 25. $\times 470$.

Fig. 27a From an embryo of the early-swimming stage; fixation in Zenker's solution; stained in iron hematoxylin; plane of section oblique vertical-horizontal; thickness, $7\ \mu$. $\times 470$.

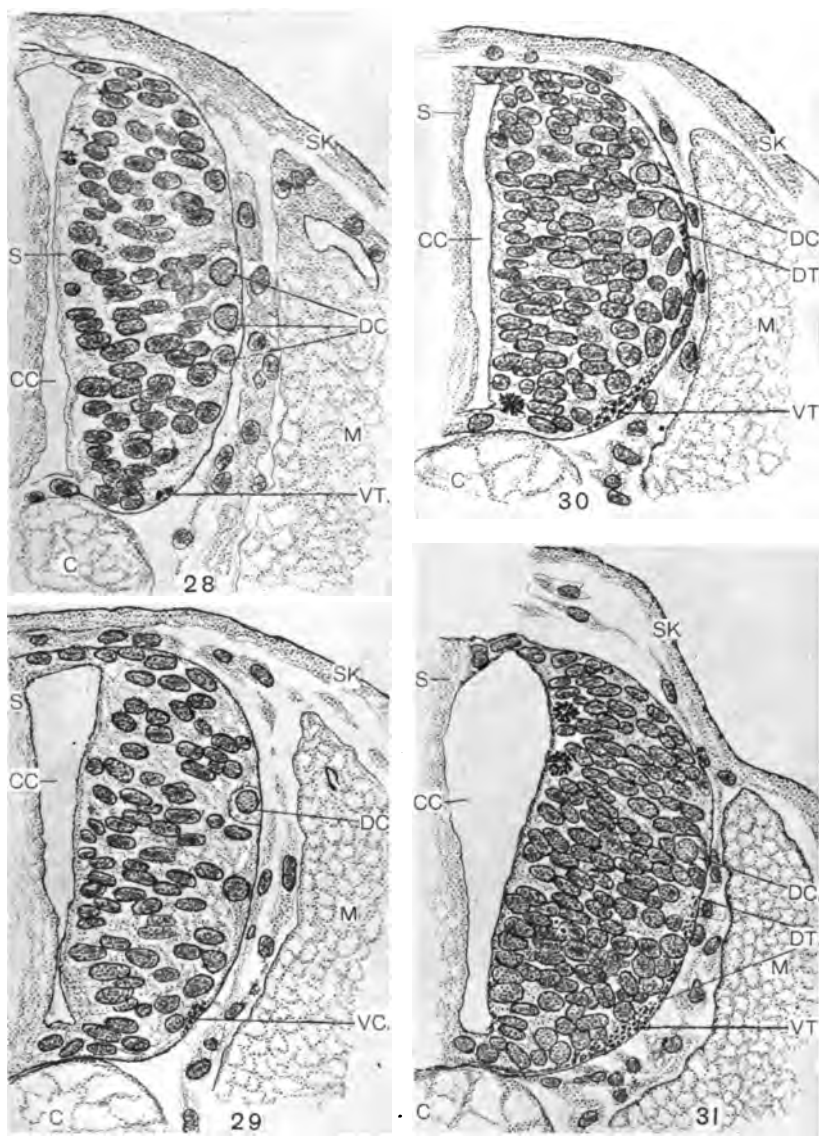
Figures 28 to 54 were drawn with the aid of the Bausch and Lomb drawing apparatus at a magnification of 400, reduced to 200 in the figures. They are taken from four embryos, designated in my series of preparations as 467, 473, 449 and 444. These embryos are taken as type specimens, respectively, of the four physiological stages that are described in the paper, namely, the non-motile stage; the early-flexure stage, the coiled stage, and the early-swimming stage. The methods of preparation of these four specimens were as follows:

467, Non-motile stage; fixation in corrosive-sublimate-acetic-acid mixture; stained in Delafield's hematoxylin and acidulated orange G; transverse plane; 10 μ .

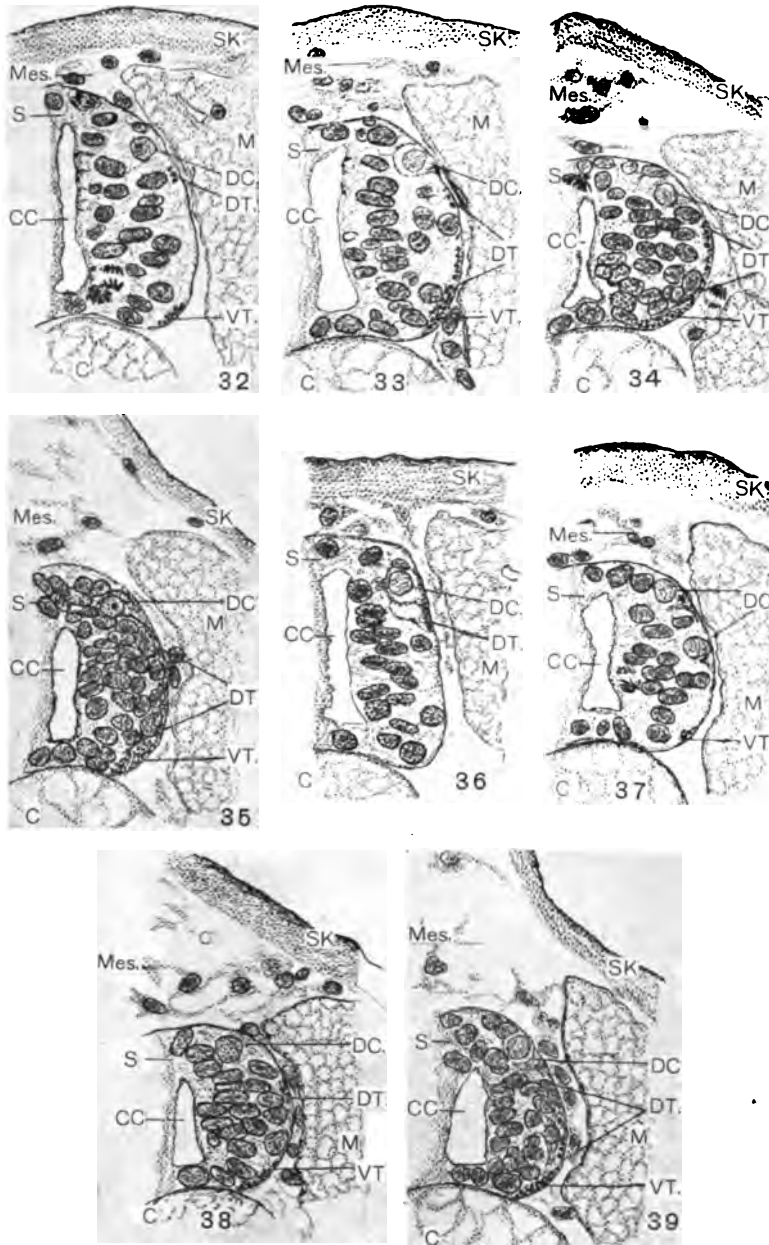
473, Early-flexure stage; fixation in corrosive sublimate-acetic acid mixture; stained in alum carmine and Lyon's blue; transverse plane; thickness, 10 μ .

449, Coil reaction stage; same fixation; stained in cochineal and Lyon's blue; transverse plane; thickness, 10 μ .

444, Early-swimming stage; same fixation; stained with Boemer's hematoxylin and acidulated orange G; transverse plane; thickness, 10 μ .

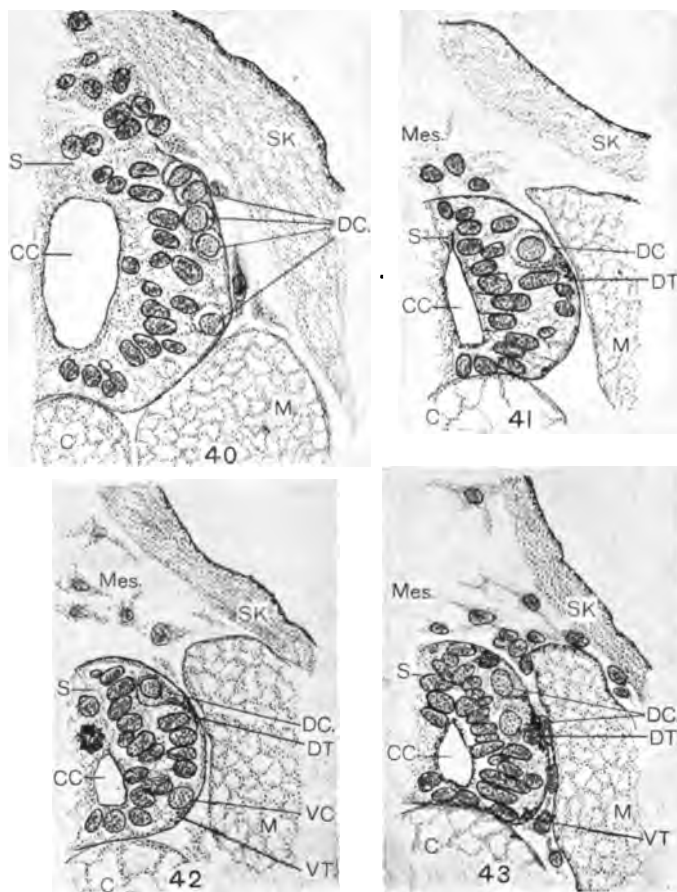


Figs. 28 to 31 From the level of the third myotome of Specimens 467, 473, 449 and 444, respectively.

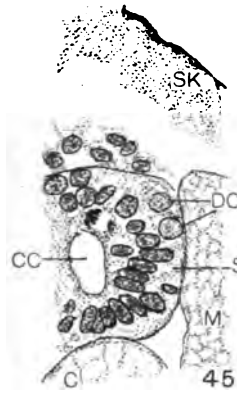
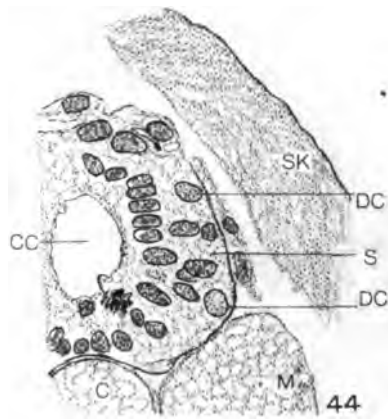


Figs. 32, 33, 34, 35 From the level of the eighth myotome of Specimens 467, 473, 449, and 444, respectively.

Figs. 36, 37, 38, 39 From the level of the thirteenth myotome 467, 473, 449 and 444, respectively.



Figs. 40, 41, 42, 43 From the level of the eighteenth myotome of the same specimens, 467, 473, 449 and 444, respectively.



Figs. 44, 45, 46, 47 From the level of the twenty-third myotome of the same specimens, 467, 473, 449 and 444, respectively.

Figs. 48, 49, 50 From the level of the twenty-eighth myotome of Specimens 473, 449 and 444, respectively.

Figs. 51, 52, 53 From the level of the thirty-third myotome of the Specimens 473, 449 and 444, respectively.

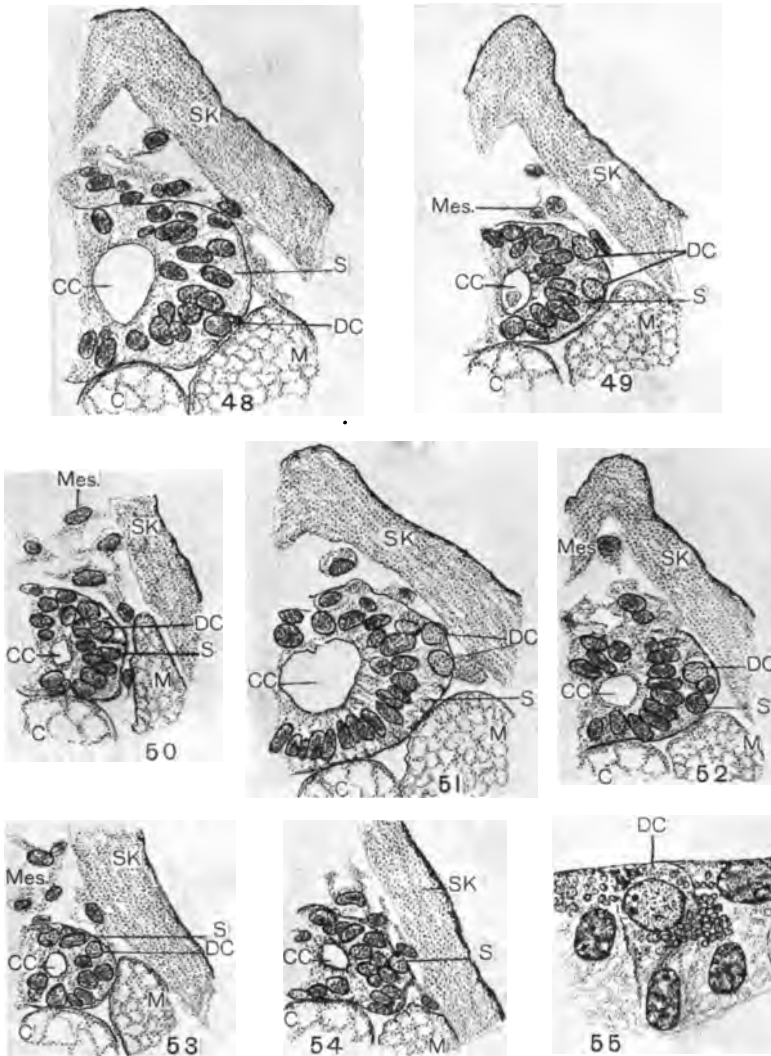


Fig. 54 From the level of the unsegmented mesoderm immediately caudal of the thirty-seventh myotome of Specimen 444.

Fig. 55 From an embryo of the non-motile stage, at the level of the root of the glossopharyngeal nerve. Transverse plane. Fixations in Van Gechuchten's alcohol-chloroform-acetic-acid solution transverse plane; thickness, 10 μ .

PLATES 1 AND 2

EXPLANATION OF FIGURES

56, 57, 58, 59 are graphic projections, made by the usual microscopical methods, at a magnification of 100 diameters (reduced in the figures to 40 diameters), from the Specimens 467, 473, 449 and 444, respectively. The methods of preparation of these specimens have been described in connection with the figures 28 to 54.

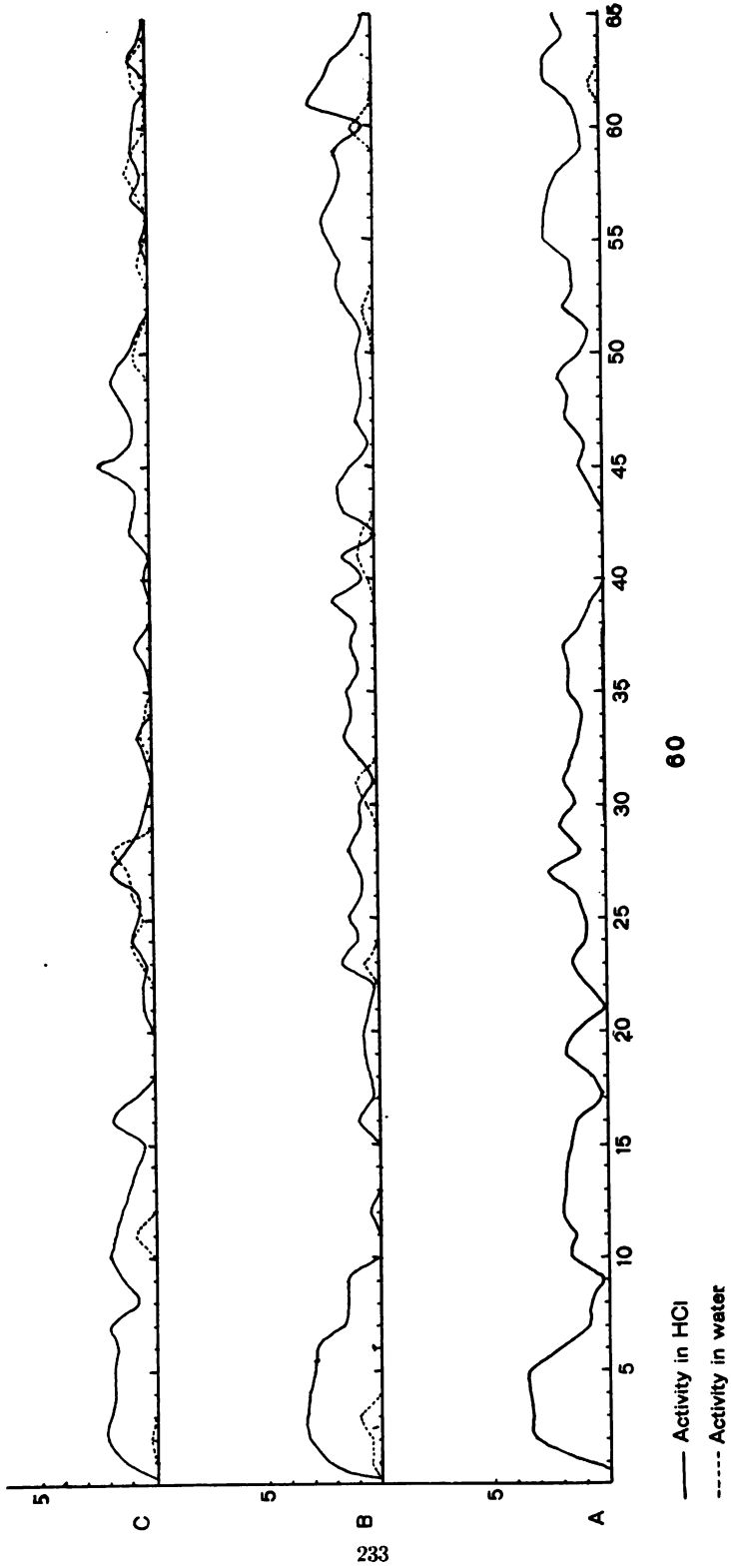
These figures show the projection of the central nervous system, the giant ganglion cells, the lateral line primordia and the ectodermal thickenings that are associated with the visceral pouches, in the four type specimens, 467, the non-motile stage, figure 56; 473, the early-flexure stage, figure 57; 449, the coil stage, figure 58; 444, the early-swimming stage, figure 59.

EXPLANATION OF FIGURE

60 Three graphs to show the activity of embryos of *Rana catesbeiana* to different dilutions of hydrochloric acid when immersed in the solution during a period of 65 seconds, as compared with their activity in water under otherwise similar conditions.

Each of these three graphs, *A*, *B* and *C*, represents the composite of the activity of five specimens. The scale on the horizontal axis indicates the time in seconds, calculated from the beginning of each experiment, when movement occurred. The scale on the vertical axis indicates in seconds the total duration of the activity of the five specimens during the successive seconds indicated on the horizontal scale. For example, in graph *A*, during the fifth second of the several experiments there was activity during a total time of about three and one-half seconds on the part of all five embryos. Again, in graph *A*, the same five embryos, during the same period of immersion in water (being handled by the same pipette and passed into the same kind of a dish as were employed in the experiments with acid) manifested perceptible activity only in the sixty-second second of the period, and there was a total activity of less than a second on the part of all five specimens.

The records upon the basis of which these graphs are constructed were made upon the rolling drum of a myograph. The recording arm of the instrument was controlled by an electro-magnet, through which the current was made and broken by the observer according to the movements of the embryos during the period. The time of the activity therefore as well as its duration was recorded for each individual specimen with a considerable degree of accuracy. The accuracy of the method depends, of course, upon the experience of the observer. In my records on the behavior of amphibian embryos are over two hundred such myograms, from which the thirty for these graphs have been selected because they were taken in rapid succession upon embryos of the same lot of eggs, and therefore represent embryos in very nearly the same age. In the activity in water, however, there is evidence of an increasing tendency to move spontaneously, the record being taken in the order of the graphs, *A*, *B*, *C*.





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FURTHER STUDIES ON THE DEVELOPMENT OF THE CRANIAL SYMPATHETIC GANGLIA

ALBERT KUNTZ

St. Louis University School of Medicine

FOUR PLATES

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INTRODUCTION

Following an extended investigation of the development of the sympathetic nervous system in the vertebrate series,¹ in which the sympathetic ganglia in the cranial region were not specifically considered, the writer has undertaken an investigation of the development of the cranial sympathetic ganglia in types of the

¹ See bibliography.

several classes of vertebrates. Observations on the development of these ganglia in embryos of the pig are published in an earlier paper.² The present paper embodies the results of a comparative study of the development of the cranial portion of the sympathetic nervous system in embryos of the common toad fish (*Opsanus tau*), the frog and *Amblystoma*, the turtle, the chick, and the pig.

In the general investigations of the development of the sympathetic nervous system, with which are associated the names of not a few able investigators, the cranial portion of that system has been quite generally neglected. Observations on the development of the ciliary ganglion in types of the several classes of vertebrates have been recorded by not a few investigators, many of whom studied the development of this ganglion in connection with their investigations of the development of the eye-muscle nerves. Recorded observations on the development of the remaining sympathetic ganglia in the cranial region, in the lower vertebrates, are fragmentary and incomplete.

A systematic review of the literature bearing on the development of the cranial portion of the sympathetic nervous system will not be attempted in this paper. In his paper on "The development of the oculomotor nerve, the ciliary ganglion, and the abducent nerve in the chick,"³ Carpenter ('06) has given us a more or less complete review of the literature bearing on the development of the ciliary ganglion in types of the several classes of vertebrates. This review was cited by the writer in the earlier paper referred to above. Recorded observations will be further considered in connection with the presentation of the results of the present investigation.

THE FISH

Introductory

The topographical relationships of the cranial sympathetic ganglia and nerves were described by Stannius in Belone as early as 1849. More recently Herrick ('99) described the topographical relationships of the cranial sympathetic ganglia and the larger

² Jour. Comp. Neur., vol. 23, pp. 71-96.

³ Bull. Mus. Comp. Zool., Harvard College, vol. 48, pp. 141, 228.

sympathetic nerves associated with them in *Menidia*. The observations of these two investigators, on two distinct types of fishes, do not differ materially and indicate that the cranial sympathetic ganglia in fishes are more or less intimately related to the roots of the X, IX, VII, V, and III cranial nerves.

The following observations on the development of the cranial portion of the sympathetic nervous system in fishes are based on preparations of embryos of the common toad fish (*Opsanus tau*). The topographical relationships of the cranial sympathetic ganglia and the sympathetic nerves associated with them, as far as the latter may be traced in preparations of embryos, conform more or less closely in this species to Herrick's description of the topographical relationships of the cranial sympathetic ganglia and nerves in adult *Menidia*. The topographical relationships of the cranial portion of the sympathetic trunk and the cranial sympathetic ganglia to the first spinal and the X, IX, VII, V, and III cranial nerves in an advanced embryo of *Opsanus* are illustrated semidiagrammatically in figure 1. This figure indicates a less intimate relationship between the sympathetic trunk and the IX cranial nerve than Herrick found to exist in *Menidia*.

Ganglia on cranial portion of sympathetic trunk

The cranial portion of the sympathetic nervous system arises, in embryos of *Opsanus*, quite as early as do the sympathetic ganglia and nerves in the trunk region. In embryos 5 to 6 mm. in length, the sympathetic trunk may be traced cephalad as far as the ganglionic complex of the trigeminal nerve. The primordia of the several sympathetic ganglia along the course of the cranial portion of the sympathetic trunk are already present.

In conformity with the plan adopted by other authors, we shall refer to the sympathetic ganglia in the course of the cranial portion of the sympathetic trunk by number, beginning with the one most anterior, which is intimately associated with the Gasserian ganglion, and numbering caudad from 1 to 6. In tracing the development of these ganglia it will be found most convenient to begin with the one most posterior in the series and consider the successive ganglia in order.

The sixth sympathetic ganglion arises in contact with the mesial aspect of the first spinal nerve and the jugular ganglion of the vagus nerve. In transverse sections of embryos of *Opsanus* 6 mm. in length, the anlage of this ganglion may be observed stretching ventro-mesially from the first spinal nerve just peripheral to the spinal ganglion (fig. 2, *sy6*). This ganglionic anlage appears, in tranverse section, as an elliptical aggregate of cells removed by a short interval from the spinal nerve, but connected with the latter by a broad cellular tract. Medullary cells may be traced, at this stage, from the mantle layer in the neural tube across the marginal veil and the external limiting membrane into the ventral root of the first spinal nerve (fig. 2, *vr spI*). As these cells advance peripherally along the fibers of the ventral nerve-root some of them mingle with the cells in the distal portion of the spinal ganglion; most of them, however, may be traced along the ventro-mesial aspect of the spinal ganglion into the path of the spinal nerve. The spinal ganglion is not sharply limited peripherally, but cells become separated from it and advance farther peripherally along the fibers of the dorsal root of the spinal nerve. Some of these cells, doubtless, deviate from the path of the spinal nerve and enter the anlage of the sympathetic ganglion. It is probable that cells which wander out from the neural tube along the fibers of the ventral nerve-root also enter the anlage of this ganglion. This sympathetic ganglion, therefore, probably receives cells from both the neural tube and the first spinal ganglion.

The anlage of the sixth sympathetic ganglion may be traced cephalad from the level of the first spinal nerve as a somewhat flattened aggregate of cells to a level somewhat anterior to the posterior margin of the ganglionic complex of the vagus nerve. In transverse sections at this level, a broad band of cells may be traced from the jugular ganglion into the sixth sympathetic ganglion (fig. 3, *sy 6*). Cells, doubtless, advance from the jugular ganglion along this tract into the sympathetic anlage. The sixth sympathetic ganglion is, therefore, genetically related to both the first spinal and the X cranial nerves.

As development advances, the sixth cranial sympathetic ganglion becomes more compact and somewhat farther removed from the first spinal nerve and the cellular tract connecting the former with the latter gradually becomes smaller and finally disappears. The anterior portion of this sympathetic ganglion remains in close proximity with the jugular ganglion. In embryos of *Opsanus* 13 mm. in length, it lies in immediate contact with the latter ganglion.

From the anterior end of the sixth sympathetic ganglion, the slender sympathetic trunk may be traced cephalad along the median aspect of the ganglionic complex of the vagus nerve. A slight ganglionic enlargement, the anlage of the fifth sympathetic ganglion, occurs at the level of the second lobe of the vagus ganglion (fig. 1, *sy* 5). This ganglionic enlargement may be observed almost as early as the sympathetic trunk can be traced. It does not become conspicuous, however, and no direct connections could be found between it and either the X or the IX cranial nerves. As early as the sympathetic trunk may be traced cephalad from the sixth ganglion, its fibers are accompanied by cells obviously of nervous origin. It is probable, therefore, that the relatively small number of cells which give rise to the fifth sympathetic ganglion advance cephalad from the sixth sympathetic ganglion.

The sympathetic trunk continues cephalad from the fifth sympathetic ganglion along the ventro-mesial aspect of the root of the IX cranial nerve but removed from the latter by a short interval. Another slight ganglionic enlargement, the anlage of the fourth sympathetic ganglion, occurs on the sympathetic trunk at the level of the ganglion of the IX cranial nerve (fig. 1, *sy* 4). This ganglionic enlargement also may be observed almost as early as the sympathetic trunk can be traced. Like the fifth sympathetic ganglion, it does not become conspicuous and no connections could be observed between it and the IX cranial nerve. It is probable, therefore, that, like the fifth sympathetic ganglion, this ganglion also arises from cells which advance cephalad from the sixth sympathetic ganglion.

The morphological relationship of the sympathetic trunk to the IX cranial nerve in *Opsanus*, as above described, differs materially from the morphological relationship of the sympathetic trunk to this nerve in *Menidia*, as described by Herrick. In the latter species, according to Herrick, the sympathetic trunk, immediately cephalad of the fifth sympathetic ganglion, fuses with the root of the IX cranial nerve, in which it may be traced as a separate fiber-tract to the ganglion of this nerve. Just before reaching this ganglion the sympathetic trunk again emerges from the root of the IX nerve and continues cephalad along its dorsal surface. The fourth sympathetic ganglion lies in close proximity with the dorsal surface of the ganglion of the IX cranial nerve.

In the fishes in which the relationships of the sympathetic trunk to the IX cranial nerve described by Herrick in *Menidia* obtain, this nerve, doubtless, plays a part in the development of the cranial sympathetic ganglia. Furthermore, the relationship of the sympathetic trunk to the IX cranial nerve here set forth in *Menidia* is probably more typical of teleosts than the relationship above described in *Opsanus*.

From the fourth sympathetic ganglion, the sympathetic trunk continues cephalad along the ventro-mesial aspect of the ganglionic complex of the VII cranial nerve. The third sympathetic ganglion arises at the level of the origin of the hyomandibular division of the VII nerve. In transverse sections of newly hatched embryos, the anlage of this ganglion appears as a slight accumulation of cells on the ventro-mesial aspect of the geniculate ganglion in proximity with the origin of the hyomandibular nerve (fig. 4, *sy 3*). This accumulation of cells is intimately associated with the geniculate ganglion and is obviously derived more or less directly from the latter. In embryos 6 mm. in length, this aggregate of cells has become completely separated from the periphery of the geniculate ganglion but still lies in close proximity with it (fig. 5, *sy 3*). It now lies in the path of the sympathetic trunk.

The second sympathetic ganglion arises at the level of the posterior portion of the Gasserian ganglion. In transverse sec-

tions of embryos 6 mm. in length, the anlage of this ganglion appears as a group of cells pushing out ventro-mesially from the periphery of the Gasserian ganglion along the wall of a large blood vessel lying in close proximity with the latter (fig. 6, *sy 2*).

The anlage of this sympathetic ganglion remains broadly connected with the Gasserian ganglion for some time. As development advances, the cells in this ganglion become more closely aggregated and the cellular tract by which it is connected with the Gasserian ganglion becomes relatively narrow. In embryos 10 mm. in length, the second sympathetic ganglion is almost completely separated from the Gasserian ganglion but remains in close proximity with it (fig. 7, *sy 2*). This ganglion, doubtless, arises more or less directly from the Gasserian ganglion. The second and third sympathetic ganglia, however, are not entirely distinct during embryonic development. As early as the sympathetic trunk may be traced at this level, numerous sympathetic cells are present among its fibers between these two ganglia. It is not impossible, therefore, that the second sympathetic ganglion may be genetically related to both the V and VII cranial nerves. The great majority of its cells, however, are, doubtless, derived directly from the Gasserian ganglion.

Cephalad of the second sympathetic ganglion, the sympathetic trunk soon enters the first sympathetic ganglion which is intimately associated with the anterior portion of the Gasserian ganglion. In transverse sections of embryos 6 to 10 mm. in length taken through the anterior region of the Gasserian ganglion, the anlage of the first sympathetic ganglion may be observed on the ventro-mesial surface of the Gasserian ganglion. During the early stages of development, the sympathetic portion of the ganglionic mass can hardly be distinguished. In the earliest stages, the cells destined to become differentiated into sympathetic ganglion cells are identical in appearance with the cells of the cerebro-spinal ganglia. As development advances, the cells of the cerebro-spinal ganglia increase in size more rapidly than do the sympathetic cells. The sympathetic anlage associated with the Gasserian ganglion may, therefore, be recognized by the

smaller size of the cells composing it. In embryos 12 to 14 mm. in length, many of the cells in the Gasserian ganglion have already become differentiated into neuroblasts. The difference in the size of the two types of cells has now become so marked that the sympathetic ganglion may be readily recognized (fig. 8, *sy 1*). It is now differentiated into a median and a lateral lobe. This ganglion obviously arises directly from the Gasserian ganglion and remains intimately associated with the latter.

Ciliary ganglion

The anlage of the ciliary ganglion arises about the time of hatching as an accumulation of cells in the path of the oculomotor nerve. In embryos 6 mm. in length, the oculomotor nerve may be traced from its origin in the wall of the mesencephalon to the anlage of the ciliary ganglion in one or two transverse sections. Emerging from the wall of the mesencephalon it advances laterally for a short distance and then makes a sharp curve ventrally. The anlage of the ciliary ganglion now lies lateral to the growing nerve and in immediate contact with it (fig. 9, *cil g*). At the point where the sharp curve occurs, there is a slight accumulation of cells obviously of nervous origin. As development advances, this accumulation of cells gradually decreases in size until it probably disappears. Throughout the entire length of the nerve-trunk its fibers are accompanied by numerous cells of nervous origin. These cells, doubtless, have their origin in the wall of the mesencephalon and advance peripherally along the fibers of the nerve-root. While medullary cells may rarely be observed in contact with or crossing the external limiting membrane, there can be no doubt that such cells wander out into the root of the oculomotor nerve in considerable numbers. Cells push out from the oculomotor nidulus in a cone-shaped mass into the root of the nerve as it traverses the marginal veil and similar cells are always present in considerable numbers in the nerve-root just outside the external limiting membrane as well as in the path of the nerve-trunk. These cells are identical in appearance with the cells in the anlage of the ciliary ganglion.

As development advances, the distal portion of the oculomotor nerve is carried cephalad. In the later stages of development, therefore, this nerve curves antero-ventrally toward the orbit. The anlage of the ciliary ganglion still lies in immediate contact with its lateral surface.

During early development the ciliary ganglion is associated solely with the oculomotor nerve. As development advances, a slender ramus composed of fibers which emerge from the first sympathetic ganglion and the anterior border of the Gasserian ganglion grows cephalad and communicates with the ciliary ganglion. This fibrous ramus which constitutes the radix ciliaris longa, however, does not communicate with the ciliary ganglion until the anlage of that ganglion has become well established. The ciliary ganglion, therefore, obviously arises primarily from cells which advance peripherally along the oculomotor nerve. After this ganglion becomes connected with the Gasserian ganglion and the first sympathetic ganglion through the radix longa, it, doubtless, receives cells which wander out from these latter ganglia along this fibrous tract. Such cells, however, must obviously be relatively few in number.

THE AMPHIBIA

Introductory

Amphibian larvae have been favored objects of study in the investigations of the development of the sympathetic nervous system, yet little is known concerning either the development or the anatomical relationships of the cranial portion of the sympathetic system in this class of vertebrates. Amphibian larvae afford less favorable material for the study of the development of the sympathetic nervous system than do the embryos of many other vertebrates. This division of the nervous system is relatively feebly developed in both the Anura and the Urodela; consequently, the sympathetic material present in their larvae is relatively meager. This is even more apparent in the cranial than in the trunk region.

Cranial extension of sympathetic trunk

In larvae of the frog 10 mm. in length or in larvae of *Amblystoma* of corresponding age, the sympathetic trunks are already well established. However, no fibrous ramus can as yet be traced cephalad from the superior cervical ganglion. In larvae of the frog 13 to 15 mm. in length, a fibrous process may be traced cephalad from the superior cervical ganglion to the level of the Gasserian ganglion. It extends along the ventro-mesial aspect of the vagus ganglion, then tends mesially and continues cephalad approximately parallel with the cerebro-spinal axis. No ganglionic enlargements occur in the course of this fibrous process nor were any aggregates of cells which could be interpreted as sympathetic ganglia observed associated with any of the cranial nerves except the oculomotor.

According to Camus ('12), no distinct sympathetic ganglia associated with the sympathetic trunk are present in the frog anterior to the superior cervical ganglion. According to this author, the fibrous process extending cephalad from the superior cervical ganglion may be traced, in larvae 13 mm. in length, as far as the level of the Gasserian ganglion. The fibers of this process decrease in number as it continues cephalad; some of them mingle with the fibers of the abducens nerve, others enter the ophthalmic division of the trigeminal nerve.

My observations on larvae of *Rana* and *Amblystoma* add nothing, regarding the extent of the cranial sympathetic nerves and the distribution of their fibers, to the findings of Camus referred to above. Like the latter author, I am also of the opinion that no distinct sympathetic ganglia associated with the sympathetic trunk are present in the *Amphibia* anterior to the superior cervical ganglion. Neither could cell-aggregates which could be interpreted as sympathetic ganglia be observed farther peripherally in the cephalic region. As is well known (Strong '95), sympathetic ganglion cells occur, in the *Amphibia* associated with certain of the cranial nerves. Such cells, however, could not be traced with certainty in my preparations.

Ciliary ganglion

The existence of a ciliary ganglion in the Urodela is somewhat doubtful. Herrick ('94) found no trace of a ciliary ganglion in *Amblystoma punctatum*. According to Coghill ('02), the ciliary ganglion in *Amblystoma tigrinum* is "transitory and probably at no time functional." Norris ('06) found no ciliary ganglion in *Amphiuma*. McKibben ('13) reports that he found no "group of cells corresponding to the ciliary ganglion" in *Necturus*.

The oculomotor nerve arises, in larvae of *Amblystoma*, from the ventro-lateral aspect of the mesencephalon as a slender strand of fibers accompanied by relatively few large cells. In larvae 13 mm. in length, this nerve may be traced from its origin into the orbit where it terminates among aggregates of mesenchymal cells which represent an early stage in the differentiation of the eye-muscles. Near the tip of the growing nerve may be observed a few large nuclei apparently identical with the nuclei of the cells accompanying the fibers of the nerve-trunk. The number of cells aggregated at this point does not increase materially as development advances and no fibrous connection could be observed between them and the Gasserian ganglion. A permanent ciliary ganglion is probably not developed in this species.

In larvae of the frog the oculomotor nerve is somewhat larger than in larvae of *Amblystoma* and a larger number of cells becomes aggregated at its growing tip. In transverse sections taken at the level of the origin of the oculomotor nerve in early larvae, medullary cells may be frequently observed pushing from the mantle layer in the wall of the mesencephalon into the root of the oculomotor nerve as it traverses the marginal veil. Similar cells are present in the oculomotor nerve just outside the external limiting membrane as well as along the entire course of the nerve. It is probable, therefore, that medullary cells advance peripherally along the oculomotor nerve and that such is the origin of the cells which become aggregated in the path of the nerve and give rise to this ganglionic enlargement which, in larvae of the frog, probably represents the anlage of a permanent ciliary ganglion.

During early development this ganglionic anlage is associated solely with the oculomotor nerve. As development advances it lies in close proximity with the anterior division of the Gasserian ganglion and probably receives fibers from the latter. This ganglionic anlage, therefore, sustains the same genetic relationships to the oculomotor and the trigeminal nerves in larvae of the frog as does the anlage of the ciliary ganglion in embryos of the fish.

Discussion

It may be noted in passing that Camus, in the paper cited above, maintains that the cells which give rise to the sympathetic ganglia have not an ectodermal origin, as is held by neurologists almost universally, but are differentiated in situ from the mesoderm. Referring to his own observations on the development of the ganglia of the sympathetic trunks in larvae of the frog, he says, "die obigen Beobachtungen zeigen vielmehr dass die sympathischen Ganglienzellen an Ort und Stelle aus dem Mesenchym sich differenziert und mit den Spinalnerven zunächst nichts zu tun haben."

This is recognized at once as a reappearance of the old theory of the mesodermal origin of the sympathetic nervous system first advanced by Remak⁴ in 1847 and later revived by Paterson⁵ ('90). It would seem to the writer hardly worth while to reconsider a theory so obviously erroneous and so long since outgrown. However, the conclusions of Camus are based on careful observations and may, therefore, merit some consideration.

As indicated above, amphibian larvae do not afford favorable material for the study of the development of the sympathetic nervous system by reason of the relatively meager sympathetic supply and the presence, in early larvae, of a large amount of yolk material. In my own work on the development of the sympathetic nervous system in the Amphibia, the pictures presented by Camus might readily have been duplicated. In sections of early larvae of *Amblystoma* or *Rana*, sympathetic cells having

⁴ On the independent alimentary nervous system. Berlin.

⁵ Phil. Trans. Royal Society., vol. 191, pp. 159-186.

no obvious relationship to the spinal nerves may be frequently observed in proximity with the aorta where the sympathetic trunk arises. It is not incredible, therefore, that a student of Goette should have concluded that these cells are differentiated in situ from the mesoderm. However, the fact that sections of early larvae may not show a direct relationship of the cells constituting the anlage of the sympathetic trunk to the spinal nerves does not afford conclusive evidence that no such relationship exists. The earliest fibers of the communicating rami are exceedingly delicate and might readily escape notice except in the most successful preparations. Furthermore, it is not impossible that cells might deviate from the course of the spinal nerves and wander toward the aorta in advance of the growing fibers of the communicating rami.

By careful study of good preparations, as the writer has shown in a series of earlier papers, cells may unmistakably be traced peripherally along the spinal nerves and into the primordia of the ganglia of the sympathetic trunks. Some of these cells retain their capacity for cell division after they have become separated from the cerebro-spinal nervous system and undergo mitosis along the path of migration or in the primordia of the sympathetic ganglia. These phenomena, it should be stated, may be more readily demonstrated in embryos of vertebrates of other classes than in larvae of the Amphibia.

The genetic relationship of the ganglia of the sympathetic trunks to the cerebro-spinal nervous system will not be further considered at this time. The cumulative evidence in favor of this view afforded by the work of not a few investigators seems to the writer conclusive.

THE TURTLE

Ciliary ganglion

The most complete observations on the development of the ciliary ganglion in a reptilian type which have been recorded are those of Béraneck and Hoffmann. Both these investigators studied the development of this ganglion in embryos of *Lacerta agilis*.

According to Béraneck ('84), the ciliary ganglion arises in intimate association with the oculomotor nerve and later becomes connected with the ophthalmic division of the trigeminal nerve. He expresses no opinion, however, as to the source of the cells taking part in its development.

According to Hoffmann ('85), the anterior part of the neural crest gives rise to two ganglia, viz., the mesocephalic ganglion associated primarily with the ophthalmic division of the trigeminal nerve, and the Gasserian ganglion. A large mass of cells, according to this author, becomes separated from the peripheral end of the mesocephalic ganglion and advances along the path of the fibrous ramus extending from the latter toward the oculomotor nerve to a point in proximity with the latter nerve. This aggregate of cells gives rise to the ciliary ganglion.

According to the writer's observations on embryos of the loggerhead turtle (*Thalassochelys caretta*), the anlage of the ciliary ganglion arises as an aggregate of cells in the path of the oculomotor nerve. This nerve arises from the ventral aspect of the mesencephalon by a fan-shaped root arranged longitudinally (fig. 11, *oc*). Its fibers are accompanied by numerous cells obviously of nervous origin. Such cells are closely aggregated in the root of the nerve outside the external limiting membrane, while within the wall of the mesencephalon cells push out from the mantle layer in cone-shaped masses into the nerve-root as it traverses the marginal veil. The cells advancing peripherally along the oculomotor nerve, therefore, are obviously cells which have wandered out from the wall of the mesencephalon, or the direct descendants of such cells. As these cells advance peripherally many of them become aggregated in the path of the oculomotor nerve and give rise to the ciliary ganglion.

In embryos of eight days incubation, the anlage of the ciliary ganglion appears as an elliptical aggregate of cells in the path of the oculomotor nerve near its growing tip (fig. 11, *cilg*). No connection can be found, at this stage, between the anlage of the ciliary ganglion and the ophthalmic division of the trigeminal nerve. In embryos of nine days incubation, a slender fibrous ramus aris-

ing from the ophthalmic nerve may be traced into the anlage of the ciliary ganglion (fig. 12, *rl*). The fibers of this ramus are accompanied by numerous nervous elements which have advanced peripherally from the Gasserian ganglion along the fibers of the ophthalmic nerve. Many of these cells enter the anlage of the ciliary ganglion. At this stage of development, however, the ciliary ganglion is already well established. It, therefore, arises primarily from cells which advance peripherally from the mesencephalon along the oculomotor nerve but later receives cells also which wander out from the Gasserian ganglion along the ophthalmic nerve.

As development advances, the cells in the anlage of the ciliary ganglion become more numerous and more compactly aggregated. The ganglion, however, remains closely associated with the oculomotor nerve.

Observations on the development of the ciliary ganglion in embryos of *Chelydra* agree in all essential respects with the observations on the development of this ganglion in *Thalassochelys* above recorded.

Sphenopalatine ganglion

The sphenopalatine ganglion arises, in embryos of the turtle, ventral and somewhat mesial to the path of the maxillary nerve. In embryos of *Chelydra* 11 to 13 mm. in length, the anlage of this ganglion appears, in sagittal sections, as an elongated aggregate of cells in the path of the great superficial petrosal nerve and connected with the maxillary nerve by one or more slender rami (fig. 15, *sph g*).

In early embryos of both *Thalassochelys* and *Chelydra*, the great superficial petrosal nerve may be traced from the geniculate ganglion into the maxillary region as a relatively large nerve whose fibers are accompanied by cells obviously of nervous origin. Near its proximal end this nerve is joined by fibers which emerge from the sympathetic plexus surrounding the carotid artery. It is not impossible that cells advancing cephalad from the superior

cervical ganglion may enter the path of the great superficial petrosal nerve in association with these fibers. However, the majority of the cells which advance peripherally along this nerve obviously wander out from the geniculate ganglion. Some of these cells become aggregated in the path of the nerve and give rise to the anlage of the sphenopalatine ganglion. Cells of nervous origin also advance peripherally from the Gasserian ganglion along the maxillary nerve. Many of these cells, doubtless, wander out along the branches of the maxillary nerve and enter the sphenopalatine ganglion. This ganglion, therefore, arises primarily from cells which advance peripherally from the geniculate ganglion along the great superficial petrosal nerve, but receives cells also which advance peripherally from the Gasserian ganglion along the maxillary division of the trigeminal nerve.

In advanced embryos of *Chelydra*, the anlage of the sphenopalatine ganglion is relatively large and irregular in outline. It retains an intimate relationship with the great superficial petrosal nerve and remains connected with the maxillary nerve by relatively slender rami. From the distal aspect of the sphenopalatine ganglion, slender nerves may be traced toward the olfactory epithelium. Figure 13, illustrating the relationships of the sphenopalatine ganglion to the maxillary and the great superficial petrosal nerves in an advanced embryo of *Chelydra*, is reconstructed from several sagittal sections.

Other ganglia

No trace of a ganglion homologous with the otic ganglion, so well developed in mammals, was observed in embryos of either *Thalassochelys* or *Chelydra*. Small aggregates of cells obviously of nervous origin were observed, in a few instances, in embryos of *Chelydra*, in the maxillary region, associated with the mandibular division of the trigeminal nerve. The destiny of these cells could not be determined. In no instance, however, was an aggregate of cells observed, in embryos of the turtle, which could be interpreted as the anlage of a submaxillary ganglion.

THE CHICK

Ciliary ganglion

Detailed observations on the development of the ciliary ganglion in the chick are recorded by Carpenter ('06) in the paper referred to in an earlier section of this paper. Earlier observations on the development of this ganglion in birds are reviewed also by this author and will not be further considered in the present paper.

According to Carpenter's observations, the ciliary ganglion arises in embryos of the chick during the fourth day of incubation as a slight accumulation of cells in the path of the oculomotor nerve. The fibers of the oculomotor nerve are, from their earliest appearance, accompanied by cells obviously of nervous origin. Carpenter has designated these elements as 'accompanying' cells and interprets them as cells of medullary origin which have wandered out from the mantle layer in the wall of the mesencephalon into the root of the oculomotor nerve and advanced peripherally along its fibers. Some of these cells become aggregated in the path of the oculomotor nerve and give rise to the anlage of the ciliary ganglion. Many of the "accompanying" cells undergo division by mitosis along the path of the nerve as well as in the anlage of the ciliary ganglion. During the fifth day of incubation, many of the cells in this ganglionic anlage become differentiated into neuroblasts. Some of them give rise to supporting elements.

During the earlier stages of development, according to Carpenter, the ciliary ganglion is not connected with the ophthalmic division of the trigeminal nerve and receives cells only via the oculomotor nerve. As development advances, cells which have advanced peripherally from the Gasserian ganglion along the ophthalmic nerve advance into the ciliary ganglion along the path of the fibrous ramus which grows from the ophthalmic nerve into the latter. The number of cells which enter the ciliary ganglion from this source, however, is relatively small. The ciliary ganglion, therefore, arises primarily from cells which advance peripherally from the wall of the mesencephalon along the

oculomotor nerve, but receives also a relatively small number of cells which wander out from the Gasserian ganglion along the path of the ophthalmic nerve.

My own observations on the development of the ciliary ganglion in the chick substantiate those of Carpenter in all essential details. Furthermore, this ganglion sustains the same genetic relationships to the oculomotor and the ophthalmic nerves in the chick as it does in the turtle and the pig and arises in essentially the same manner in embryos of these three types of vertebrates. Detailed observations on the development of the ciliary ganglion in embryos of the pig are recorded in an earlier paper. Observations on the development of this ganglion in embryos of the turtle are recorded in an earlier section of the present paper. Detailed observations on the development of this ganglion in embryos of the chick will, therefore, not be presented in this paper.

Otic ganglion

In embryos of the chick in which the sympathetic trunks are becoming well differentiated, a fibrous process may be traced cephalad from the superior cervical ganglion along the median aspect of the carotid artery until it merges into the sympathetic plexus surrounding this artery. At the level of the geniculate ganglion fibers again emerge from this plexus. Some of these fibers mingle with the fibers of the great superficial petrosal nerve; the majority of them, however, continue cephalad, forming a more or less definite fiber-tract. A relatively small number of fibers from the great superficial petrosal nerve also enters this tract. A few cells with nuclei of somewhat larger size than the nuclei of the surrounding cells may be observed in the sympathetic plexus surrounding the carotid artery. These cells are, doubtless, sympathetic elements which have advanced cephalad from the superior cervical ganglion. Similar cells are present in the fiber-tract which continues cephalad from the plexus surrounding the carotid artery. Some of these cells, doubtless, advance peripherally from the geniculate ganglion along the fibers which deviate from the course

of the great superficial petrosal nerve and enter this tract. The majority of the nervous elements in this fiber-tract, however, probably are cells which have advanced cephalad from the superior cervical ganglion.

As early as the fibers emerging from the sympathetic plexus surrounding the carotid artery may be traced cephalad, the cells of nervous origin accompanying them are relatively numerous. Some of these cells soon become aggregated into a ganglionic mass which represents the anlage of the otic ganglion. This ganglionic anlage arises but a short distance cephalad of the geniculate ganglion. As development advances, it becomes removed somewhat farther cephalad until it lies mesial to and slightly caudad of the origin of the mandibular division of the trigeminal nerve. Figure 16 illustrates the relationships of the otic ganglion to the great superficial petrosal nerve and the sympathetic plexus surrounding the carotid artery, as observed in sagittal sections of an embryo of the chick during the seventh day of incubation. The relationships of the otic ganglion to the superior cervical ganglion and the VII and V cranial nerves are illustrated diagrammatically in figure 17.

No fibrous or cellular communications could be observed, during the early stages of development, between the otic ganglion and the mandibular division of the trigeminal nerve or the Gasserian ganglion. The cells which take part in its development are derived primarily from the superior cervical and the geniculate ganglia.

Sphenopalatine ganglion

The great superficial petrosal nerve may early be traced, in embryos of the chick, from the geniculate ganglion into the maxillary region where it terminates in the loosely aggregated anlage of the sphenopalatine ganglion in proximity with the olfactory epithelium (fig. 18, *sphg*). This ganglion receives fibrous communications also from the maxillary division of the trigeminal nerve. This division of the trigeminal is a relatively slender nerve containing relatively few cells of nervous origin. There is no

evidence that any considerable number of cells advancing peripherally along the fibers of this nerve enter the anlage of the sphenopalatine ganglion. The fibers of the great superficial petrosal nerve, on the other hand, are accompanied by numerous cells of nervous origin, many of which obviously enter this ganglion.

In view of the above observations, the sphenopalatine ganglion arises, in embryos of the chick, primarily from cells which advance peripherally from the geniculate ganglion along the great superficial petrosal nerve, but probably receives cells also which advance peripherally from the Gasserian ganglion along the maxillary nerve. In view of its genetic relationships to the great superficial petrosal and the maxillary nerves, this ganglion is homologous with the sphenopalatine ganglion in the turtle, but is not entirely homologous with the sphenopalatine ganglion in the pig.

Submaxillary ganglion

The submaxillary ganglion is only relatively feebly developed in the chick. It arises in the submaxillary region in the path of a slender branch of the mandibular division of the trigeminal nerve. (fig. 19, *submg*). Neither sympathetic root nor fibrous connection of the submaxillary ganglion with the facial nerve could be observed during the early stages of development. The submaxillary ganglion is, therefore, genetically related primarily to the mandibular nerve and probably arises exclusively from cells which advance peripherally along the fibers of this nerve.

THE PIG

Summary

According to the writer's observations set forth in the earlier paper referred to above, the ciliary, the sphenopalatine, the otic, and the submaxillary ganglia arise, in embryos of the pig, primarily from cells which have their origin in the Gasserian ganglion and the walls of the mesencephalon and rhombencephalon and advance peripherally along the oculomotor nerve and the several

divisions of the trigeminal nerve. These cells, like the cells which give rise to the ganglia of the other parts of the sympathetic nervous system, have their origin in a cerebro-spinal ganglion, i.e., a ganglion which is derived from the neural crest, and in motor noduli in the walls of the neural tube and advance peripherally along sensory and motor nerve-fibers respectively.

The ciliary ganglion, as indicated above, bears the same genetic relationships to the oculomotor and the ophthalmic nerves and arises in essentially the same manner in embryos of the pig as in embryos of the turtle and the chick.

The sphenopalatine ganglion arises, in embryos of the pig, primarily from cells which advance peripherally from the Gasserian ganglion along the maxillary nerve. It becomes connected with the geniculate ganglion through the great superficial petrosal nerve but probably receives relatively few cells from this source. This ganglion does not bear the same genetic relationship to the great superficial petrosal and the maxillary nerves respectively in the pig as does the ganglion described above as the sphenopalatine ganglion in the turtle and the chick.

The otic ganglion arises, in embryos of the pig, at the mesial surface of the proximal portion of the mandibular division of the trigeminal nerve primarily from cells which advance peripherally from the Gasserian ganglion and the wall of the rhombencephalon respectively along the sensory and motor roots of this nerve. This ganglion receives a slender sympathetic root but probably few or no cells enter it from this source. The genetic relationships of the otic ganglion in the pig differ widely from those of the ganglion described above as the otic ganglion in the chick.

The submaxillary ganglion arises, in embryos of the pig, in the submaxillary region in proximity with the lingual division of the mandibular nerve from cells which advance peripherally along this nerve. This ganglion bears the same genetic relationship to the mandibular nerve in the pig as in the chick.

DISCUSSION AND CONCLUSIONS

According to the observations set forth in the preceding pages, the cranial sympathetic ganglia bear the same genetic relationships to the cerebro-spinal nervous system, in all the classes of vertebrates, as do the ganglia of the other parts of the sympathetic nervous system, i.e., they arise from cells which have their origin in the cerebro-spinal ganglia and the wall of the neural tube and advance peripherally along sensory and motor nerve-roots respectively. Not all the nervous elements taking part in the development of the sympathetic ganglia actually migrate as such from the cerebro-spinal ganglia or the neural tube; many of them arise by the mitotic division of cells which have advanced peripherally from the cerebro-spinal nervous system.

The character and the destiny of the cells which become separated from the cerebro-spinal nervous system and advance peripherally along the cranial and spinal nerves have been discussed by the writer in earlier papers and will, therefore, not be considered in detail at this time. The majority of these elements are cells of the 'indifferent' type, many of which retain the capacity for cell division after they have become separated from the cerebro-spinal ganglia or the neural tube. Consequently, mitotic figures may be frequently observed along the paths of migration and in the peripheral ganglia. Such mitotic division of nervous elements in the paths of peripheral nerves and the primordia of peripheral ganglia probably occurs less frequently in embryos of the lower than in embryos of the higher vertebrates. Neither do all the elements arising in this manner take part in the development of sympathetic ganglia. Many of them obviously become differentiated into cells of the neurilemma.

The distribution and the relative degree of development of the cranial sympathetic ganglia varies greatly in the several classes of vertebrates. The degree of development of the several cranial sympathetic ganglia is obviously correlated with the demands of the functions of the structures innervated by the sympathetic nerves associated with them.

SUMMARY

1. The sympathetic ganglia on the cranial portion of the sympathetic trunks arise, in embryos of the toad fish, primarily from cells derived directly from the first spinal ganglion and the cerebral ganglia associated with the X, VII, and V cranial nerves. Certain of these ganglia receive cells also which advance peripherally from the neural tube along motor nerve-roots. The ciliary ganglion arises in the path of the oculomotor nerve primarily from cells which advance peripherally from the mesencephalon along this nerve. It later receives a relatively small number of cells which advance peripherally from the Gasserian ganglion and the first sympathetic ganglion associated with the latter along the radix ciliaris longa.

2. A permanent ciliary ganglion is probably not developed in *Amblystoma*. In larvae of the frog, the ciliary ganglion arises in essentially the same manner as in embryos of the fish. Other distinct cranial sympathetic ganglia probably do not occur in the Amphibia.

3. In embryos of the turtle, the ciliary ganglion arises in the path of the oculomotor nerve primarily from cells which advance peripherally from the mesencephalon along this nerve. After this ganglion has become connected with the ophthalmic nerve it receives a relatively small number of cells which advance peripherally from the Gasserian ganglion.

The sphenopalatine ganglion arises, in embryos of the turtle, in the path of the great superficial petrosal nerve and soon becomes connected by fibrous rami with the maxillary nerve. It arises from cells which advance peripherally from the geniculate and the Gasserian ganglia respectively along the great superficial petrosal and the maxillary nerves.

Ganglia homologous with the otic and the submaxillary ganglia of the higher vertebrates were not observed in embryos of the turtle.

4. In embryos of the chick, the ciliary ganglion bears the same genetic relationships to the oculomotor and the ophthalmic

nerves and arises in essentially the same manner as in embryos of the turtle.

The otic ganglion arises, in embryos of the chick, in the path of a tract of sympathetic fibers which emerge from the sympathetic plexus surrounding the carotid artery, primarily from cells which are derived from the superior cervical and the geniculate ganglia.

The sphenopalatine ganglion arises, in embryos of the chick, primarily from cells which advance peripherally from the geniculate ganglion along the great superficial petrosal nerve, but probably receives cells also which advance peripherally from the Gasserian ganglion along the maxillary nerve.

The relatively small submaxillary ganglion is genetically related to the mandibular nerve.

5. In embryos of the pig, as set forth in an earlier paper, the ciliary ganglion is genetically related to the oculomotor and the ophthalmic nerves. The sphenopalatine ganglion arises from cells which advance peripherally from the Gasserian ganglion along the maxillary nerve. The otic and the submaxillary ganglia are genetically related to the mandibular division of the trigeminal nerve and probably receive cells from both the Gasserian ganglion and the wall of the rhombencephalon.

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ABBREVIATIONS

<i>ca</i> , carotid artery	<i>nX, nIX, nVII, nV, nIII, X, IX, VII,</i> <i>V, III</i> cranial nerves
<i>cil g</i> , ciliary ganglion	<i>nt</i> , neural tube
<i>erm</i> , epithelium of roof of oral cavity	<i>oc</i> , oculomotor nerve
<i>fb</i> , fore-brain	<i>ole</i> , olfactory epithelium
<i>fm</i> , floor of oral cavity	<i>oph</i> , ophthalmic nerve
<i>gX</i> , ganglionic complex of X cranial nerve	<i>or</i> , orbit
<i>gIX</i> , ganglion of IX cranial nerve	<i>otg</i> , otic ganglion
<i>gVII</i> , ganglionic complex of VII cranial nerve	<i>pc</i> , sympathetic plexus surrounding carotid artery
<i>gV</i> , Gasserian ganglion	<i>rl</i> , radix ciliaris longa
<i>Gasg</i> , Gasserian ganglion	<i>rlX</i> , ramus lateralis vagi
<i>gsp</i> , great superficial petrosal nerve	<i>rm</i> , roof of oral cavity
<i>hb</i> , hind-brain	<i>scg</i> , superior cervical ganglion
<i>jugg</i> , jugular ganglion	<i>spgI</i> , first spinal ganglion
<i>man</i> , mandibular nerve	<i>sphg</i> , sphenopalatine ganglion
<i>manb</i> , branch of mandibular nerve	<i>submg</i> , submaxillary ganglion
<i>mb</i> , mid-brain	<i>sy1, sy2, sy3, sy4, sy5, sy6, sy7</i> , 1, 2, 3, 4, 5, 6 sympathetic ganglia
<i>max</i> , maxillary nerve	<i>vr, spI</i> , ventral root of first spinal nerve
<i>maxb</i> , branch of maxillary nerve	

PLATE 1

EXPLANATION OF FIGURES

- 1 Diagram illustrating the topographical relationships of the cranial sympathetic ganglia to the first spinal and the X, IX, VII, V, and III cranial nerves in an advanced embryo of the toad fish.
- 2 Transverse section at level of first spinal nerve and sixth sympathetic ganglion, embryo of toad fish 6 mm. in length. $\times 300$.
- 3 Transverse section at level of jugular ganglion and anterior portion of sixth sympathetic ganglion, embryo of toad fish 6 mm. in length. $\times 300$.
- 4 Transverse section at level of third sympathetic ganglion, newly hatched embryo of toad fish. $\times 300$.
- 5 Transverse section at level of third sympathetic ganglion, embryo of toad fish 6 mm. in length. $\times 300$.
- 6 Transverse section at level of second sympathetic ganglion, embryo of toad fish 6 mm. in length. $\times 300$.
- 7 Transverse section at level of second sympathetic ganglion, embryo of toad fish 10 mm. in length. $\times 300$.

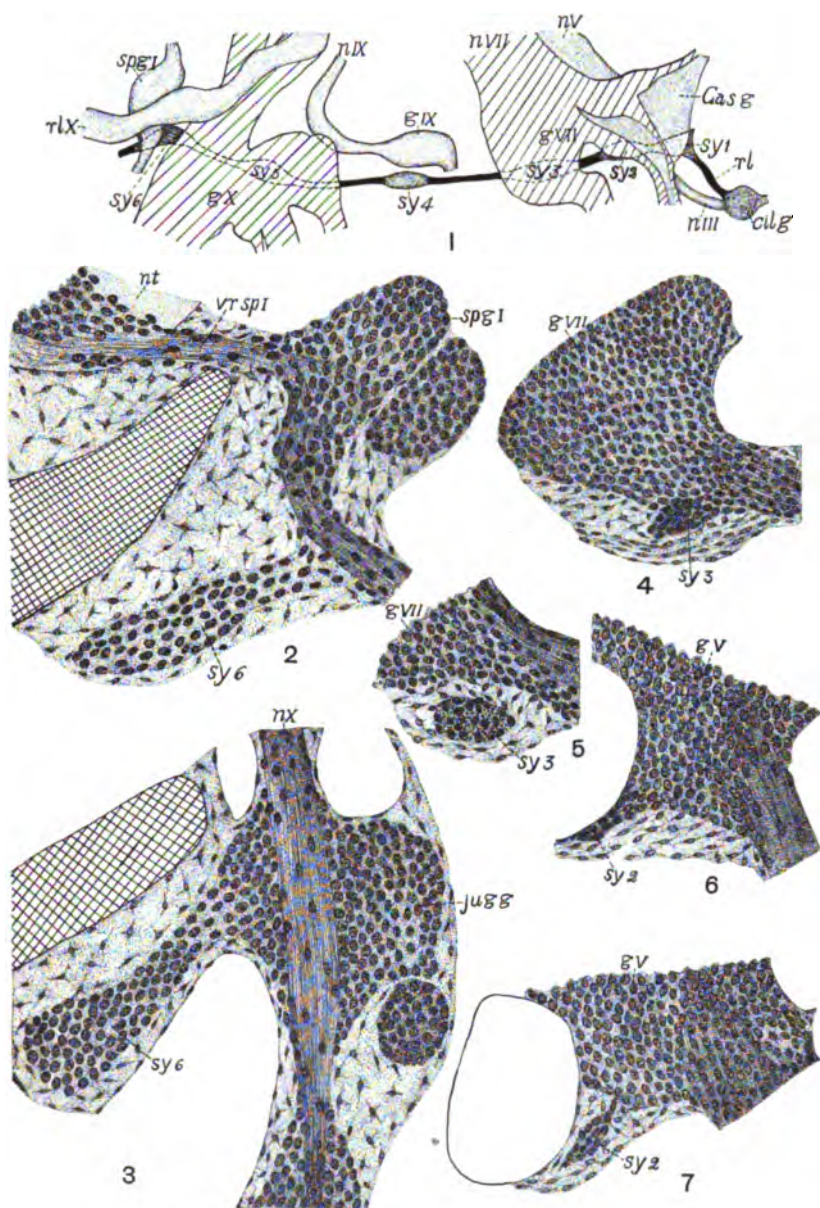


PLATE 2

EXPLANATION OF FIGURES

8 Transverse section through Gasserian and first sympathetic ganglia, embryo of toad fish 14 mm. in length. $\times 300$.

9 Transverse section at level of oculomotor nerve, embryo of toad fish 6 mm. in length. $\times 115$.

10 Transverse section at level of oculomotor nerve, larva of *Amblystoma* 13 mm. in length.

11 Sagittal section showing oculomotor nerve, 8 day embryo of loggerhead turtle. $\times 65$.

12 Sagittal section showing ophthalmic nerve and ciliary ganglion, 9 day embryo of loggerhead turtle. $\times 90$.

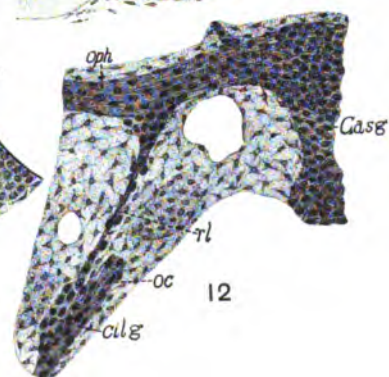
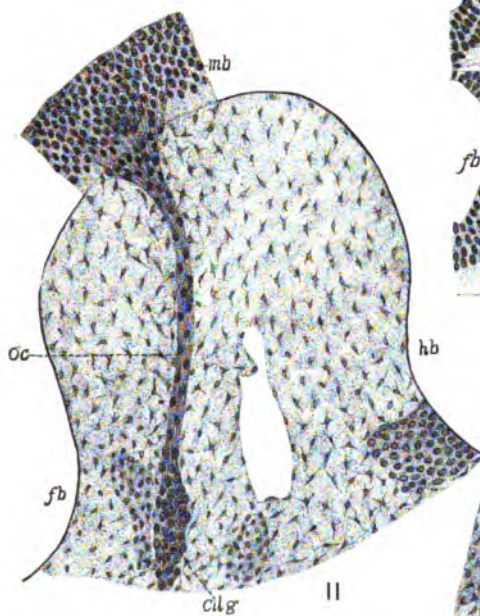
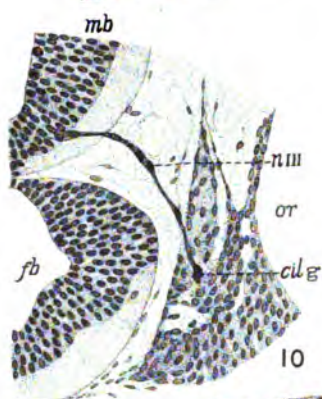
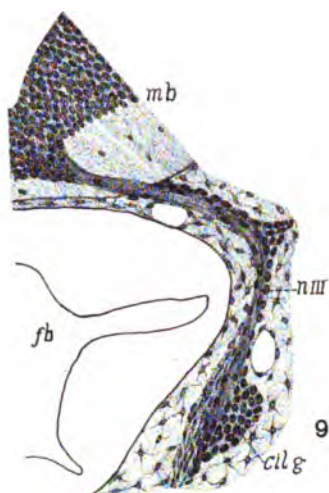
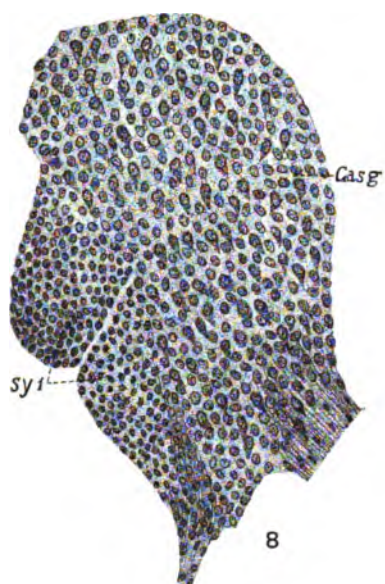


PLATE 3

EXPLANATION OF FIGURES

13 Sphenopalatine ganglion reconstructed from several sagittal sections of advanced embryo of Chelydra. $\times 65$.

14 Diagrammatic reconstruction showing topographical relationships of ciliary and sphenopalatine ganglia to cranial nerves, embryo of Chelydra 13 mm. in length.

15 Sagittal section through sphenopalatine ganglion, embryo of Chelydra 14 mm. in length.

16 Sagittal section through otic ganglion, 156 hour embryo of chick. $\times 75$.

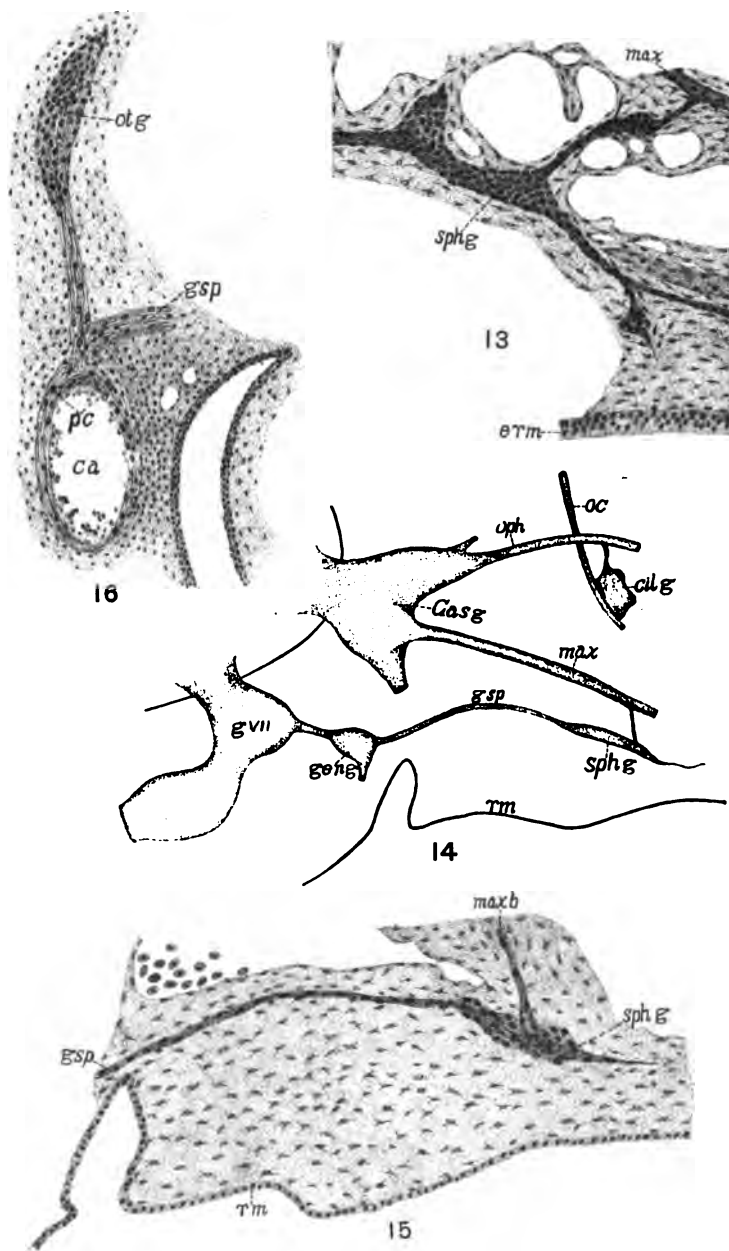


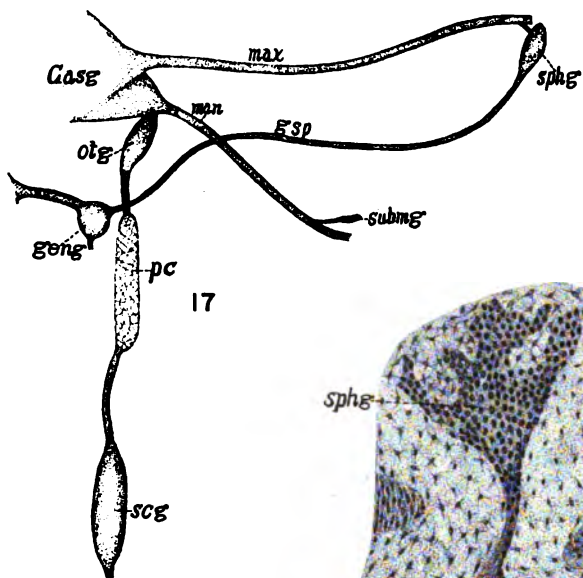
PLATE 4

EXPLANATION OF FIGURES

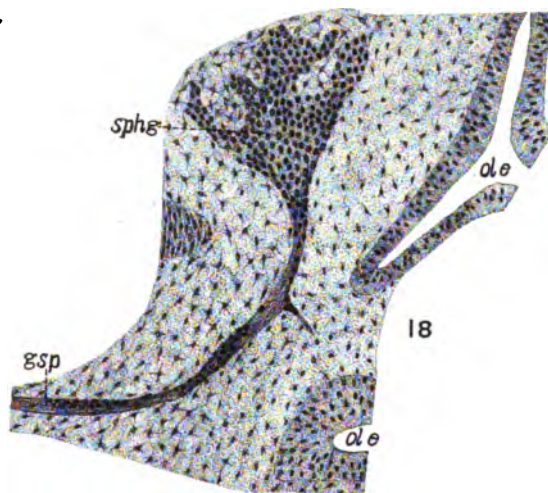
17 Diagrammatic reconstruction illustrating topographical relationships of sphenopalatine, otic, and submaxillary ganglia to superior cervical ganglion and cranial nerves, 156 hour embryo of chick.

18 Sagittal section through sphenopalatine ganglion, 168 hour embryo of chick. $\times 90$.

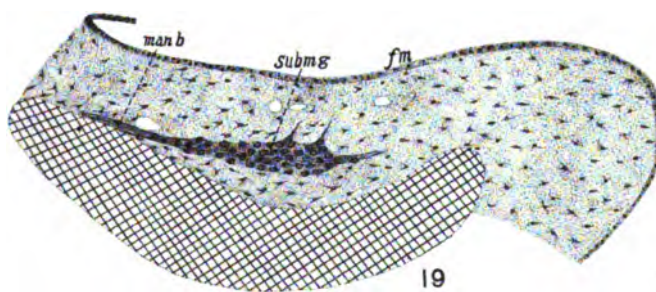
19 Sagittal section through submaxillary ganglion, 168 hour embryo of chick.



17



18



19

A STUDY OF GANGLION CELLS IN THE SYMPATHETIC NERVOUS SYSTEM, WITH SPECIAL REFERENCE TO INTRINSIC SENSORY NEURONES

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From the Zoological Laboratory, University of Illinois,¹ and from the Biological Laboratory of Trinity College, Hartford, Connecticut

TWENTY-TWO FIGURES

The existence in the sympathetic nervous system of intrinsic sensory neurones—that is, afferent neurones whose cell-bodies lie in autonomic ganglia—is still one of the unsettled questions of neurology. The conflicting statements found in present day textbooks of human anatomy reflect the lack of agreement on this point among investigators. On the one hand it is held, in conformity with the views of Kölliker and Langley, that all afferent fibers present in the sympathetic nerves are the peripheral processes of neurones whose cell-bodies are found in the spinal ganglia. Such special dorsal-root neurones are believed to send their processes via the rami communicantes into the sympathetic trunks, where they meet with and accompany the efferent post-ganglionic fibers to their distribution in tissue under control of the autonomic system. The opposing view, supported by Dogiel, while not necessarily denying the presence of afferent cerebro-spinal fibers, recognizes in the sympathetic mechanism sensory neurones which belong primarily to that system. These have their trophic centers in the various autonomic ganglia. Their peripherally directed processes terminate in sensory endings in the viscera, etc.; their centrally directed processes pass through rami communicantes into the ganglia of the dorsal nerve roots, and here form terminal arborizations about the spinal ganglion

¹ Contributions from the Zoological Laboratory, University of Illinois, under the direction of Henry B. Ward, No. 30.

cells, chiefly those of Dogiel's type II. In some cases the centripetal fibers of such neurones are believed to end within sympathetic ganglia in connection with the dendrites of efferent cells.

A considerable amount of indirect evidence in support of the latter view has accumulated from various sources. It has been repeatedly shown that during development the embryonic spinal ganglia (which later, when functional, are entirely sensory) contribute cells to the forming sympathetic ganglia. The experimental demonstration of reflexes in the domain of the sympathetic system after communication with the spinal nerves and cord has been severed seems to indicate the presence of entire local sensory elements capable of acting as the afferent arm of a reflex arc. However, it should be recalled that Langley explains such reactions by his ingenious "axon reflex" hypothesis, which takes into account only neurones that are ordinarily efferent in character. From anatomical sources comes such indirect evidence as that furnished by the comprehensive study of the elements of spinal ganglia made by Dogiel ('08). Non-medullated fibers terminating within spinal ganglia in the form of pericellular end-nets have been assumed to come from the sympathetic system. That is to say, they are regarded as the centrally directed processes of afferent autonomic neurones, the cell-bodies of which lie in sympathetic ganglia.

With these facts pointing to the existence of intrinsic sensory neurones, we might expect to find confirmatory evidence in the direct histological examination of the tissues concerned. Can it be shown under the microscope that sensory and motor neurones with differential structural characters occur? In the cerebro-spinal system of peripheral nerves such characters are, as is well known, easily demonstrable.

A perusal of the literature seems to indicate that practically the only direct observations on what appear to be distinct sensory and motor types of sympathetic cells are those recorded by Dogiel ('96), for mammals and other vertebrates, in his article entitled, "*Zwei Arten sympathischer Nervenzellen.*" A number of authors (Cajal, Müller, Michailow) have, it is true, described different varieties of autonomic cells, Michailow recording no

less than seven; but none of these authors has clearly differentiated a distinct sensory type of cell, although Michailow regards as sensory some of the dendritic endings found by him. In general, the various forms observed have been looked upon as variations of the motor elements, due largely to differences in the character of the dendrites and in the lobation or fenestration of the cell-bodies.

The present work was undertaken to confirm, if possible, by means of the silver-nitrate and Nissl methods, the existence of Dogiel's sensory and motor types in the sympathetic system of mammals. Dogiel's description of these elements as seen after methylene blue staining will be given in connection with the account of our own observations.

SYMPATHETIC GANGLION CELLS OF THE CAT (CAJAL SILVER-NITRATE METHOD)

As Dogiel's observations were made on methylene blue material it was thought advisable to check them first with a silver impregnation method. For this purpose the silver-nitrate reduction process of Cajal was employed. One advantage of this method for the complete study of the elements of a ganglion lies in its tendency to differentiate, in successful preparations, all the cells—not merely a few here and there as is so often the case with methylene blue staining. A disadvantage, however, must be recognized in the opacity of the tissue caused by the general deposition of the reduced silver. As a result thinner sections must be cut than when methylene blue is used, in order that the individual cells may be clearly seen under the microscope. With such comparatively thin sections (10 to 20 micra) the processes of cell-bodies often cannot be followed distally as far as is desirable.

In spite of the limitations of the method, Dogiel's two types of cells can clearly be made out in Cajal preparations of the superior cervical ganglion of the cat. For instance, figure 1 is easily recognizable as his motor type, described as possessing a round, oval, fusiform or stellate cell-body, often more or less compressed, from which are given off from 5 to 20 comparatively thick and

short, branched dendrites. These cells are numerous in the various ganglia of the system. It is equally certain that his sensory type is represented by figure 2. Regarding this form of neurone Dogiel states that the cell-body may be conical or club-shaped, but never flattened; that it is usually somewhat larger than the first type; that the processes, which may number 16 or more, are finer, longer, and have fewer branches; and that the cells are not as abundant, being, in some of the smaller ganglia, altogether absent.

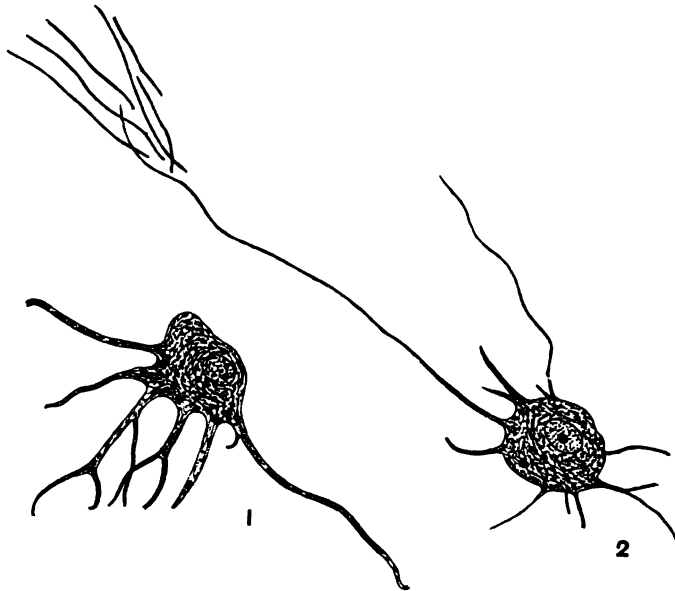


Fig. 1 Ganglion cell representing Dogiel's "motor type" from the superior cervical ganglion of the cat. Silver-nitrate preparation.

Fig. 2 Ganglion cell representing Dogiel's "sensory type" from the superior cervical ganglion of the cat. Silver-nitrate preparation.

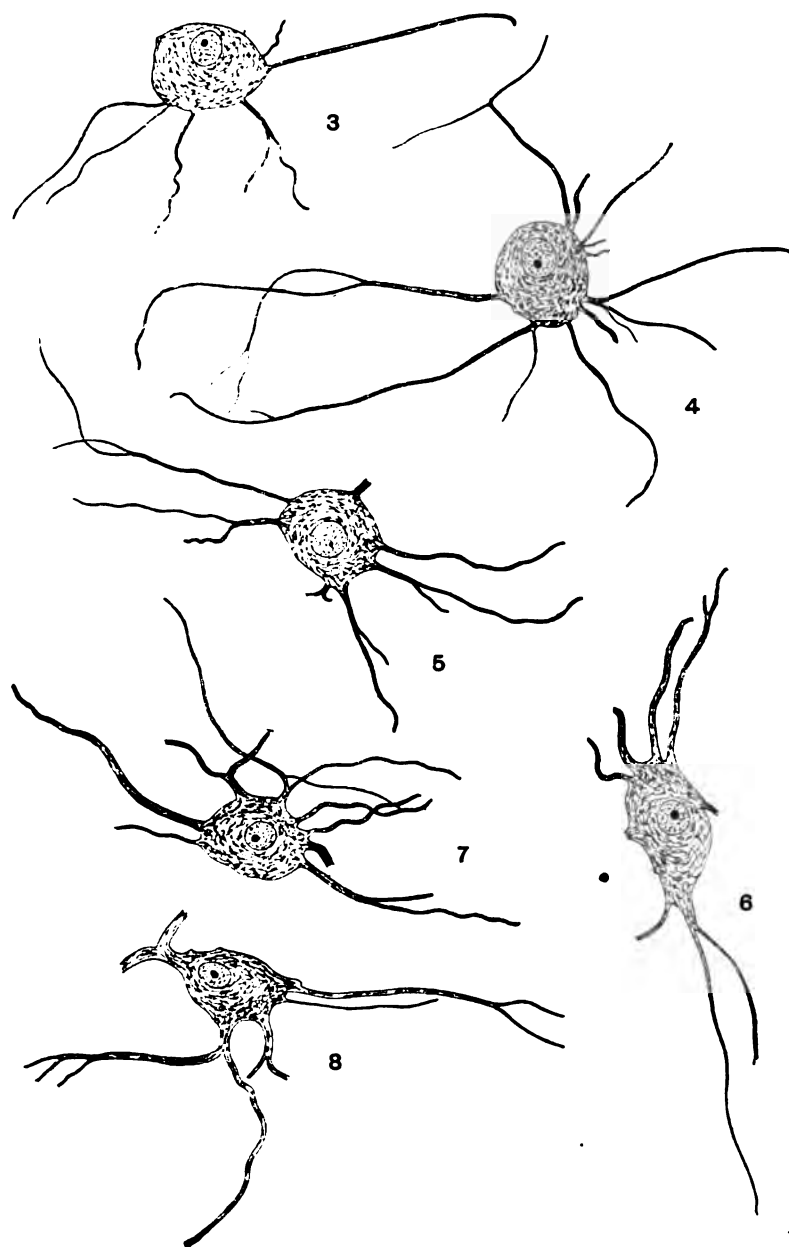
Although cells answering to the description of Dogiel's two types are present in our preparations, we are not, however, convinced that these cells are representatives of two distinct categories. If they are cells of opposite function, one being afferent and the other efferent, then we might, by analogy, expect to find them clearly differentiated structurally into two sharply delimited groups without intermediate forms. This is true of the

peripheral neurones of the cerebro-spinal system, and appears to have been Dogiel's idea when he wrote his account of the two types under consideration.

In our silver-nitrate material, which probably shows a more complete picture of the cellular elements of a single ganglion than has been obtained with methylene blue, neurones intermediate in structure between the two extremes described above are of frequent occurrence. Cells may be selected so as to form a series showing a gradual transition from one type to the other. If we start with the so-called sensory type, we may see in this series a slight but constant increase in the size and branching of the dendrites until the other extreme is reached, the so-called motor type. Cells from such a series are represented in figures 3 to 8. If we accept Dogiel's classification, it is quite impossible to assign those neurones that occupy intermediate positions to either his motor or his sensory group. Since, then, the two types are not sharply separated by morphological characters, but are, on the contrary, connected by intermediate forms, they are probably to be looked upon as extreme examples of the variation existing among the sympathetic ganglion cells, all of which may very possibly be of one function, i.e., motor. This follows from the fact that it is the presence of the sensory type only that is open to question. The morphological differences among the neurones are conceivably due, as other writers have suggested, to a correlation with differences in the kinds of tissues innervated by them.

SYMPATHETIC GANGLION CELLS OF THE RABBIT (NISSL METHOD)

The attempt to separate sympathetic cells into two precise groups having proved negative when attention was directed to their external morphology, we next undertook a study of the chromatophile substance of the cell-bodies to see if the separation might be effected on the basis of differences in internal structure. Here, again, we have in the cerebro-spinal system of nerves constant and well-defined internal characters which sharply mark off the sensory from the motor neurones. A glance at fig-



Figs. 3 to 8 Ganglion cells from the superior cervical ganglion of the cat showing transitional forms between the "sensory" and "motor types" of Dogiel. Silver-nitrate preparation.

ures 9 to 13 will show how easily recognizable these differences are. The first two drawings show sensory cell-bodies from a cranial and a spinal ganglion with their numerous, small Nissl flakes evenly distributed throughout the cytoplasm. Figures 11 to 13 represent motor cell-bodies from the nuclei of origin of ventral-root neurites in the gray substance of the spinal cord. Their less numerous and much larger chromatophile bodies are in striking contrast to those of the sensory cells. Furthermore, the recent studies of Jacobsohn ('10) and Malone ('13) show that in the central nervous system afferent and efferent cells may often be distinguished one from the other by means of the Nissl bodies, which are, in general, relatively coarser in efferent cells.

A study of Nissl preparations made from the thoracic, superior mesenteric, and coeliac ganglia of the rabbit has, however, failed to reveal to us two types of sympathetic cells. The Nissl picture presented is a very constant one (figs. 14 to 19). In general the chromatophile bodies are intermediate in size between those of sensory cerebro-spinal cells and those of motor cerebro-spinal cells. They are often massed toward the periphery of the cell, with only a few scattered flakes in the more central region around the nucleus (figs. 14, 16, 18, 19). This arrangement was found to obtain in nearly all cells which were viewed in optical section passing through their centers. Occasionally, however, cells were seen in which the peripheral grouping was not pronounced, the Nissl bodies being distributed fairly evenly throughout the cytoplasm of the cell-body (figs. 15 and 17), but these cells can hardly be said to form a distinct group. The characteristic peripheral arrangement of the Nissl flakes in the majority of sympathetic ganglion cells of the rabbit was observed by Eve ('96) in his study of the physiology of the basophile constituents.

An examination of the cranial autonomic ganglia showed us that the Nissl picture is in these cells very similar to that seen in the sympathetic cells proper. This is apparent from an inspection of figures 20, 21, and 22 which represent, respectively, elements from the otic, ciliary and sphenopalatine ganglia. Among the cranial autonomic cells the perinuclear area frequently appears even more devoid of Nissl flakes than it does among the cells of the sympathetic division of the system.

On the whole, then, the study of the Nissl bodies of autonomic ganglion cells in the rabbit has tended to convince us that, as far as these constituents are concerned, only one type of cell exists. If this means that the cells are all alike functionally, then, as pointed out above, it must be the motor type that is present.

The sympathetic cells of a number of other rodents were examined by the Nissl method, and found to present much the same appearance in respect to the chromatophile bodies as those of the rabbit. The peripheral arrangement of the flakes was a nearly constant feature, and enabled the observer to identify the cells at a glance as sympathetic. The material for this comparative study was obtained from the trunk autonomic ganglia of the rat, mouse, thirteen-lined spermophile, prairie dog, muskrat, guinea-pig and porcupine.

Although it has no direct bearing on the matter in hand, the binucleate condition of many of the rabbit's sympathetic cells offers some features of interest. Throughout the vertebral and prevertebral ganglia of the sympathetic division of the autonomic system cells with two nuclei (figs. 15 to 18) occur in great abundance. In the peripheral ganglia situated in the wall of the digestive tube (submucous and myenteric plexuses) we have not observed such cells; neither have we found them in the cranial autonomic ganglia (ciliary, sphenopalatine, otic). Our observations in regard to their presence, or rather absence, in the cranial region are in general accord with those of Apolant ('96). In order to ascertain the position of the dividing line between the binucleate cells of the trunk and the uninucleate cells of the head, we made preparations of the superior cervical ganglion and of the plexus arising from it anteriorly around the internal carotid artery. Many of the cells of the superior cervical ganglion show two nuclei each, but as soon as the carotid plexus is reached the ganglion cells scattered in it appear to be exclusively uninucleate, thus conforming to the type of the more compact autonomic ganglia of the head region, such as the ciliary, otic and sphenopalatine. The loose sympathetic ganglia of the orbit, described by Peschel ('93), also failed to show cells with two nuclei. Langley's separation of the autonomic system into cranial and

sympathetic (cervical, thoracic, lumbar) components seems in the rabbit to be justified by morphological differences in the ganglion cells, as well as by the interval which, in the central nervous axis, separates the cells of origin of the two divisions.

In the group of rodents there appears to be considerable variation in the relative abundance of these binucleate cells in the sympathetic ganglia. Apolant ('96) reported that the rabbit and guinea-pig showed them to a marked degree, while it was exceptional to find a sympathetic cell with two nuclei in the rat, mouse and squirrel. We are able to supplement Apolant's observations to the extent of increasing by two the list of rodents in which binucleate sympathetic cells are present. We have found them well represented in the muskrat and porcupine. The greatest number of such cells appears to be possessed by the rabbit and guinea-pig, with the muskrat and porcupine following in the order in which they are named. No binucleate cells were seen in the trunk autonomic ganglia of the rat, mouse, thirteen-lined spermophile and prairie dog, although it is possible that a few exist.

SUMMARY

1. Sympathetic ganglion cells of the cat prepared by the Cajal silver-nitrate method show both Dogiel's "motor type" of cell with heavy dendrites and his "sensory type" with slender dendrites. They also show cells with intermediate structural characters connecting the two "types." On the basis of external cell morphology we cannot say, therefore, that two distinct kinds of elements exist in the sympathetic ganglia. The "types" of Dogiel are, in our opinion, to be regarded as extremes of the variation which occurs among the multipolar sympathetic cells.

2. Sympathetic ganglion cells of the rabbit prepared by the Nissl method present a quite constant picture of the chromatophile bodies, which tend to be arranged near the periphery of the cell-body. The cells cannot be divided by this feature of their internal morphology into two well defined groups.

3. As far as our anatomical observations go there is nothing to indicate that ganglion cells of opposite, i.e., motor and sensory function exist in the sympathetic system. If their structural similarity is an indication of similarity in function, then all must be motor, since it is the presence of intrinsic sensory neurones only that it is open to question.

4. The sympathetic ganglion cells of the rat, mouse, thirteen-lined spermophile, prairie dog, muskrat, guinea-pig and porcupine present a Nissl picture similar to that seen in the sympathetic cells of the rabbit.

5. In the rabbit many binucleate cells were found in the vertebral and prevertebral ganglia of the trunk region (sympathetic division proper of the autonomic system). Such cells were not seen in the cranial autonomic ganglia, nor in the plexuses of the intestinal wall (peripheral ganglia).

6. The sympathetic ganglia of the guinea pig, muskrat and porcupine possess, like those of the rabbit, a considerable number of cells with two nuclei. Such cells are rarely if ever to be found in the sympathetic ganglia of certain other rodents, viz., the rat, mouse, thirteen-lined spermophile and prairie dog.

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PLATE 1

EXPLANATION OF FIGURES

All figures are of ganglion cells of the rabbit stained by the Nissl method. The drawings were made with the aid of the camera lucida. Magnification for all figures, 625 diameters.

9 Sensory cell from the Gasserian ganglion.

10 Sensory cells from the dorsal-root ganglion of a spinal nerve.

11 to 13 Motor cells from the ventral horn of the spinal cord.

14 to 17 Cells from ganglia in the thoracic region of the sympathetic trunk. Figures 14 and 16 show the characteristic peripheral arrangement of Nissl bodies seen in the majority of sympathetic cells. Figure 17 represents a cell more heavily stained than the others. As the figures show, many of the sympathetic cells are binucleate.

18 Cell from the superior mesenteric ganglion.

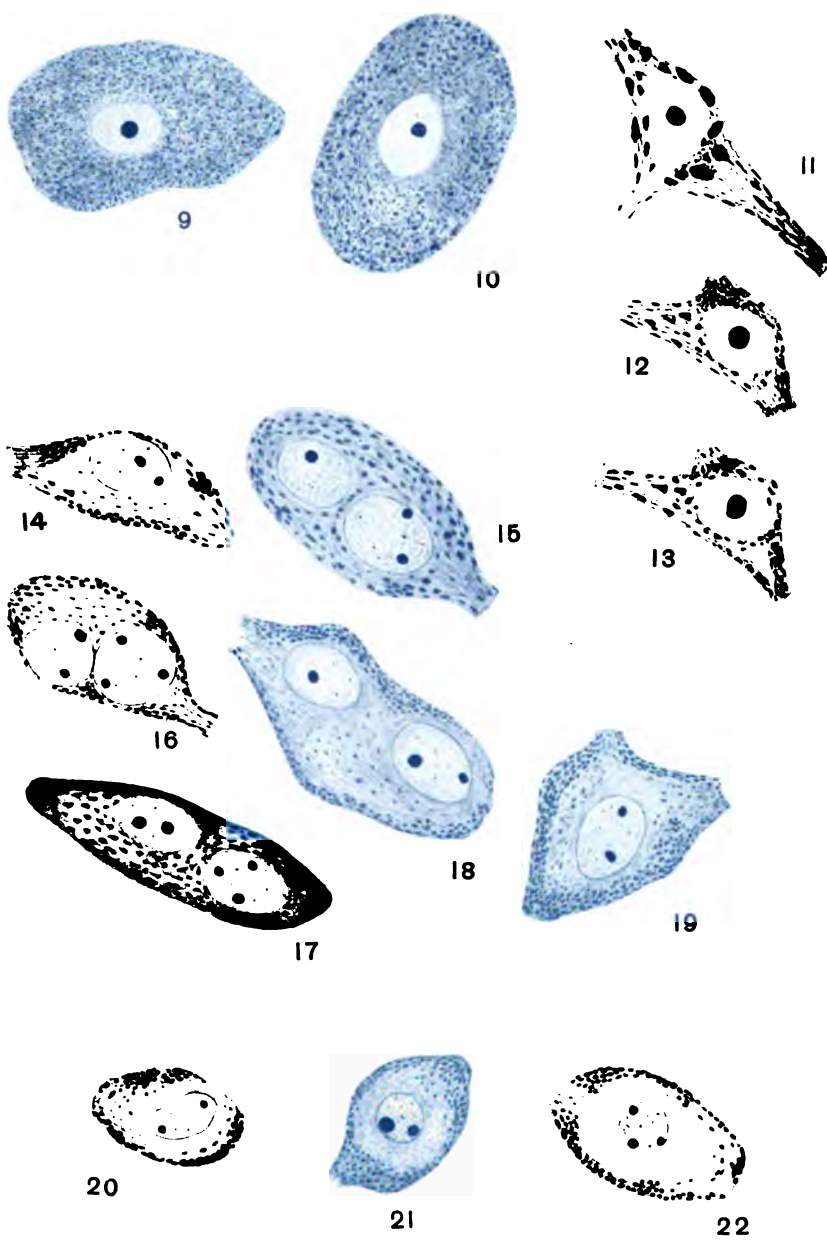
19 Cell from the coeliac ganglion.

20 Cell from the otic ganglion.

21 Cell from the ciliary ganglion.

22 Cell from the sphenopalatine ganglion.

The cells of the autonomic cranial ganglia are uninucleate, but show the peripheral arrangement of the Nissl bodies with a clear perinuclear area.



THE POSTERIOR ROOTS OF THE MUSHROOM BODIES IN THE WORKER OF BOMBUS SP.

CAROLINE BURLING THOMPSON

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EIGHT FIGURES

The present study has been made to determine whether the relation between the posterior roots of the mushroom bodies and the central body is the same in the bumble bee as in the ants previously described, Thompson, 1913.

The material, consisting of the brains of the adult workers of *Bombus* sp., was collected in August, 1913, in Wellesley, Mass. The heads were removed from the body, and before being placed in the fixative, Gilson's fluid, cuts were made in the epidermis. After fixation and hardening the heads were opened and the brains dissected out and embedded in paraffin. The sections were about 9μ thick and were stained in Ehrlich's hematoxylin and eosin.

The series of cross sections drawn in figures 1 to 8 trace the course of both the central body roots and the posterior roots of the mushroom bodies, and show the relation of these roots to the central body, to the ocellar nerve fibers, and to the posterior dorsal commissure.

Figure 1 is from a section through the posterior part of the main stalks of the mushroom bodies, *st.*, and through the posterior region of the central body, *c.b.* The two parts of the central body are shown: the upper, large and fan-shaped, consisting of masses of fibers in transverse section alternating with radial and interlacing bundles in longitudinal section; the lower part smaller, and also containing fibers that are radially arranged. Fibers may be

seen passing between the central body, and the mushroom body stalks and protocerebral tissue, *pl.*

In figure 2 the upper part of the central body is the same as in the preceeding section, but the lower part is divided by the issuing and entering fibers into two lobes or masses of fibers. The posterior surface of the mushroom body stalks is shown in this section, and fibers coming from the inner division of the stalk are seen in longitudinal section, curving up into the central body and forming the "central body roots" of the mushroom bodies, *c.b.r.* Penetrating between these root fibers are other fibers that run between the central body and the protocerebral tissue.

In figure 3 the upper part of the central body, *c.b.*, is disappearing, and consists of disconnected masses of fibers in cross section and bundles of longitudinally cut fibers that are evidently making their exit to the protocerebral tissue. The two lower lobes or fiber masses are still present on each side of the median line. Lateral to each of these are two bundles of fibers in cross section, *p.r.*, derived from the mushroom body stalks, the outermost bundles still connected by longitudinally running fibers with the inner part of the stalks. These bundles, which are issuing from the mushroom body stalks in a plane just posterior to that of the central body roots, continue backward and finally enter the protocerebral core, and are the "posterior roots" of the mushroom bodies, as will be seen from the following description. The heavier stippling of the bundles of the posterior roots represents, not larger nerve fibers, but larger bundles of fibers within the main bundle. The stalks of the mushroom bodies have nearly disappeared in this section, only the most distal portion remaining.

It is therefore evident from figures 2 and 3, that in *Bombus*, as in the ants previously described, the mushroom body stalks, or the "inner roots" of various authors, do not end abruptly beneath the central body as heretofore stated, but that their fibers divide into two bundles, namely: the central body roots, figure 2, *c.b.r.*, and the posterior roots of the mushroom bodies, figure 3, *p.r.*, which go respectively to the central body and to the posterior part of the protocerebral core.

Figure 4 shows the two heavily stippled fiber bundles of each posterior root, *p.r.*, and the lightly stippled masses of fibers derived from the central body, *c.b.f.*, some of which are passing down into the protocerebral tissue. The stalks of the mushroom bodies have entirely disappeared except for a slight remnant of the left stalk.

In figure 5 the fibers derived from the central body, *c.b.f.*, show a further diminution and now exist as two small rounded masses which are connected with each other on the dorsal surface. Fibers are still passing out of these masses into the protocerebral tissue. The two lateral bundles of the posterior roots are fused into one, *p.r.*, situated on each side of the central body fibers, *c.b.f.*, and connected with them by fibers. The proximal ends of the lateral ocellar nerves, *loc.n.*, appear in this section.

In figure 6 the fibers from each posterior root have moved up and fused with a few remaining fibers from the central body. The fibers of the posterior roots are no longer grouped into small bundles within a larger one, as was shown by the heavier stippling in figures 3, 4, 5, but are spread out at equal distances from each other, and are therefore no longer distinguishable from the fibers derived from the central body. A delicate nerve sheath with small nerve cells on its outer surfaces now surrounds each of these fiber masses, which are here termed the posterior roots, *p.r.*, even though they also contain some fibers from the central body.

In figures 7 and 8, it may be seen that the two large fiber masses, *p.r.*, or the posterior roots of the mushroom bodies containing also some fibers from the central body, move down nearer to the protocerebral core and finally merge into it in a manner similar to that in ants.

These two large masses of the posterior roots of the mushroom bodies have been frequently termed "ocellar glomeruli" in the literature of insect brains, on account of their supposed connection with the ocellar nerve fibers. Figures 6, 7 and 8 show that in *Bombus* as in the ants previously described, there is no connection between the two sets of structures, the ocellar nerves, *loc.n.*, merely passing between, never into the posterior roots, *p.r.* The term "ocellar glomeruli" is therefore a misnomer for *Bombus*.

Jonescu '09 describes, in *Apis*, fibers from the central body to the "ocellar glomeruli" which he says continue as the ocellar nerves to the ocelli. On page 142 he states:

Fasern von der Pars intercerebralis (Haller) dringen durch den Zentralkörper in die Ocellarglomerulen ein und gelangen dann weiter als Ocellarnervenfaser in die Ocellen. Charakteristisch finde ich einen Theil der Fasern, welche aus den inneren Kapseln des Zentralkörpers kommen und eine Kreuzung vor dem Eintritt in die Ocellarglomerulen bilden (siehe Frontalschnitt Fig. 22).

As stated above, there is no connection in *Bombus* or in ants between the ocellar nerve fibers and the posterior roots, the "ocellar glomeruli" of Jonescu. The characteristic fibers which Jonescu describes passing from the central body to the "ocellar glomeruli" are evidently fibers either belonging to the central body roots of the mushroom bodies, or issuing from the central body to the protocerebral tissue. The term "tubercles of the central body" as frequently applied to the two masses of the posterior roots is also inexact, although less so in *Bombus* than when applied to ants, for in *Bombus*, as was shown above, some fibers from the central body accompany the fibers of the posterior roots of the mushroom bodies. In this genus the term "tubercles of the central body" is not altogether incorrect, but is misleading, and in the opinion of the writer, should be abandoned, together with its synonym "ocellar glomeruli," in favor of the term "posterior roots of the mushroom bodies."

Figures 6, 7 and 8 show the commissure, or horizontal fiber tract connecting the right and left protocerebral lobes, which was termed by Jonescu for *Apis* the "Ocellarnervenbrücke," but which, as found in ants, has been named by the writer the "posterior dorsal commissure." This term, rather than "Ocellarnervenbrücke," should be applied to the structure found in *Bombus*, figures 6, 7, 8, *p.d.cm.*, since the ocellar nerve fibers *loc.n.*, pass to the protocerebral fibrous core through the commissure and not along it. Von Alten, 1910, text-figure 4 a, has figured the same relative arrangement of fibers in the brain of the *Bombus* queen. Jonescu, however, finds that in the honey bee the ocellar nerve fibers go to the protocerebral lobes by the way of the posterior

commissure, that is, curving out of the vertical plane, and passing to the fibrous core side by side and together with the commissure fibers, making what he aptly calls a "chiasmatische Bahn." It is not surprising that this difference should occur between *Bombus* and *Apis mellifica*, since in ants a great variety in structure exists between different genera and even between the castes of one genus, Thompson, 1913, page 531:

To summarize: in ants the ocellar nerve fibers may take three different paths from the ocellar lobes: (1) by way of the anterior dorsal commissure, queens of *Lasius* and *Camponotus*, (2) through the posterior commissure, *Formica* queen, *Formica* and *Lasius* workers, (3) both through and by way of the posterior dorsal commissure, males of the three genera.

Since the fiber tract in question is an "Ocellarnervenbrücke," or path for the ocellar nerves, only in certain cases, but is invariably the "posterior dorsal commissure," the latter term has a better excuse for existence than the former. The writer trusts that the name "Ocellarnervenbrücke" may be cast away together with "protocerebral nerve bridge," "ocellar glomeruli," and "tubercles of the central body."

The origin of the posterior dorsal commissure in *Bombus* is from the protocerebral fibrous core, figure 8, as in ants, and does not arise from cells of the intercerebral region as Jonescu states is the case in *Apis mellifica*.

February 27, 1914.

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ABBREVIATIONS

<i>c.b.</i> , central body	<i>p.d.cm.</i> , posterior dorsal commissure
<i>c.b.f.</i> , central body fibers	<i>p.r.</i> , posterior roots
<i>c.b.r.</i> , central body roots	<i>st.</i> , stalks of mushroom body
<i>loc.n.</i> , lateral ocellar nerve	

PLATE 1

EXPLANATION OF FIGURES

All figures are drawn with the Zeiss camera lucida, Zeiss oc. 2, AA, 150 mm. tube length. Figures 1 to 8 represent consecutive sections through the posterior part of the brain of the worker of *Bombus* sp. The outer nerve cell layer is blank, the fibrous portions are mottled.

1 Section through the posterior part of the mushroom bodies, showing the stalks *st.*, connected with the cups, and the posterior part of the central body, *c.b.* Fibers are seen passing between the central body and the protocerebral lobes, *p.l.*

2 Section showing fibers in longitudinal section passing from the mushroom bodies stalks into the central body, *c.b.*, and forming the central body roots of the mushroom body, *c.b.r.*

3 Section through the posterior end of the central body, *c.b.*, showing its disintegration into smaller fiber bundles, and also showing the exit of the fibers of the posterior roots, *p.r.*, from the mushroom body stalks.

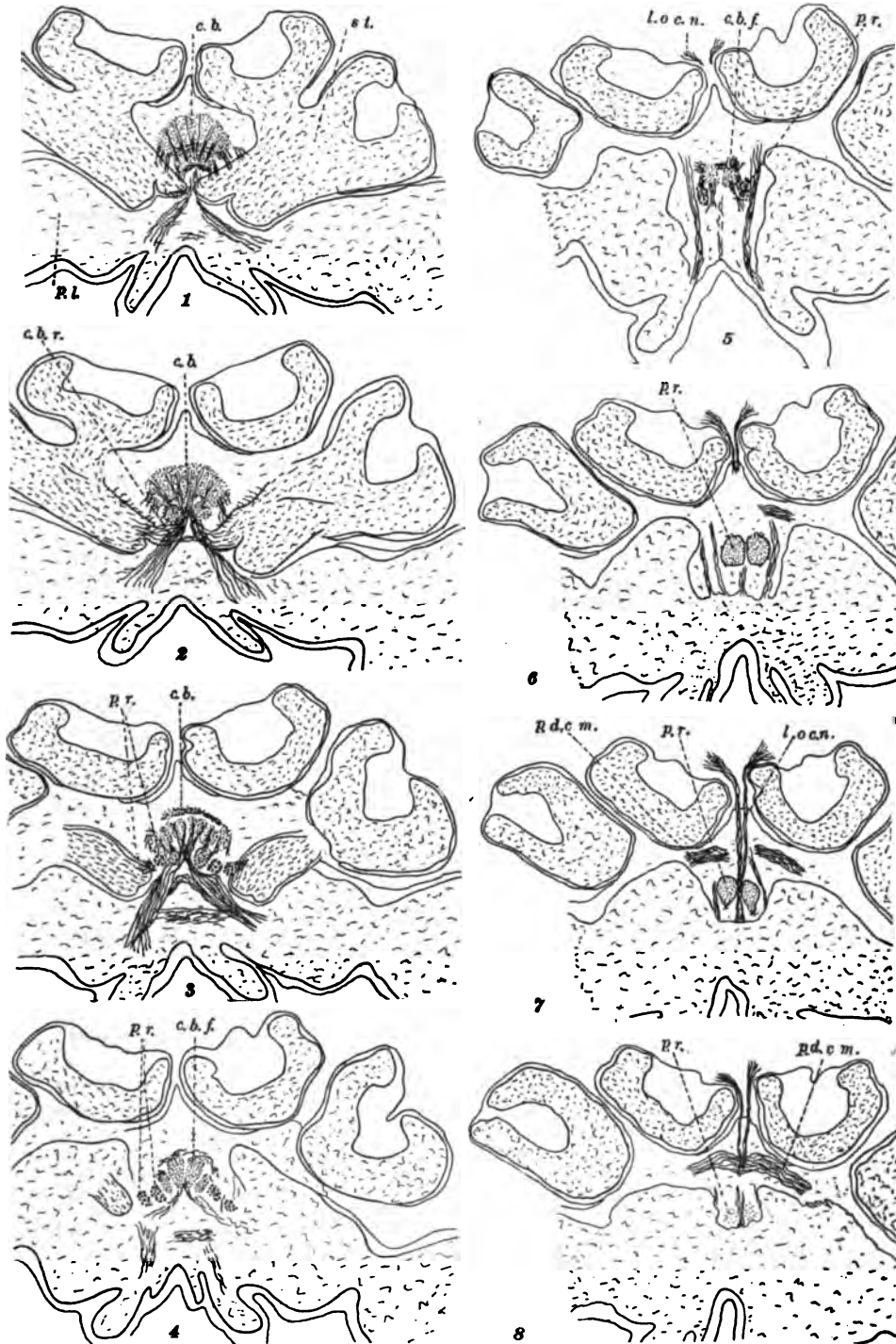
4 Section posterior to the mushroom body stalks, showing four groups of fibers in light stippling derived from the central body, *c.b.f.*, and four lateral groups in heavy stipplings, the posterior roots of the mushroom bodies, *p.r.*

5 Shows the continued passage of fibers from the remains of the central body *c.b.f.*, to the protocerebral tissue and the approach of the posterior root fibers, *p.r.* The proximal part of the lateral ocellar nerves, *loc.n.* is seen.

6 Shows the fusion of the remaining central body fibers with those of the posterior roots, *p.r.* The beginning of the posterior dorsal commissure is seen on the right.

7 The two posterior roots, *p.r.*, are moving down toward the protocerebral core. The ocellar nerve fibers, *loc.n.*, pass between the posterior roots, and the posterior dorsal commissure, *p.d.cm.*, is above them.

8 The posterior roots, *p.r.*, are merging into the protocerebral core. The origin of the posterior dorsal commissure, *p.d.cm.*, from the protocerebral tissue is seen on the right side.



THE PARIETAL REGION IN THE PRIMATE BRAIN

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NINETEEN FIGURES

During the last ten years the problems of the interpretation of the cerebral sulci and of the various areas into which they divide the cortex have come to be regarded from a new point of view. The mass of detailed observations concerning the structural localization of the cortex which has been accumulated renders it necessary, therefore, to reexamine these sulci in the light of this new information and to determine, as far as may be possible, what meaning is to be attached to the detailed findings of Vogt, Brodmann, Mauss and many others.

It is eminently desirable that such an inquiry should be undertaken in order that the facts of anatomical localization may be correlated with the varying pattern of the convolutions. This is all the more necessary because much of our information concerning cerebral localization has been collected by investigators who have minimized or even denied the influence of localization in determining the arrangement of the cerebral sulci. Our knowledge at present is, however, sufficient to indicate that the distribution of the differentiated cortical areas is the chief factor in determining the development of sulci in definite situations, either along the boundaries of areas or in the axis of a given area.

In this communication I propose to study the parietal region and the areas adjoining it in the light of this new knowledge. More especially will it concern itself with the changes produced in the inferior parietal area, changes which result from the enormous expansion and differentiation of this area during the transition from the simian to the human condition.

In the cerebral hemispheres of man, at present, there is probably no region in which it is more difficult to find fixed landmarks for comparative study than in that portion of the lateral surface of the hemisphere commonly marked off as the occipital region and those parts of the inferior parietal and temporal regions immediately in front. It is interesting or discouraging, depending upon the object in view, to compare the fissural patterns of this part of the brain as they are given in various text-books of anatomy and neurology. Almost anything to suit the taste can be found from admirable illustrations of actual brains to the most startling and schematic diagrams which might possibly represent an as yet unobserved condition. The reason for such diverse representations is to be sought in the great relative variability of this portion of the cortex and it is this variability which has led so many writers to dismiss the subject with only a few words or even to neglect it almost completely. The various attempts which have been made to mark off sharply an occipital lobe are but further evidence of the difficulties involved. One could with comparative ease find definite limits for an occipital lobe in most of the anthropoids but in man such limits must be largely arbitrary and represent no natural subdivision. From what is to follow it will be clear that the danger of insisting upon an arbitrary delimitation of various parts of the cerebral hemisphere should not be underestimated.

Variability, wherever it is found, suggests at once, if it exceeds rather narrow limits, that the organ or structure concerned has not yet reached its full development or else that it has long since passed that point and is on the wane. That portion of the neopallium under discussion at present would fall into the former class in which, as it were, a condition of comparative equilibrium has not yet been established. The same holds true for many other parts of the body and investigators have not shrunk from repeated attempts to solve the problems involved in these, but in the case of the brain it would seem as if the prospect of a satisfactory explanation were too remote to tempt to any detailed investigation.

Difficulties of such a character must always be interesting and it is the hope of throwing some light upon those of the parietal region of the brain that this work is undertaken.

Comparative anatomy rather than embryology must form the groundwork upon which to build for the reason that the structures in question are relatively new, in a phylogenetic sense. The only near approach to the complex conditions obtaining in man is found in the highest of the Simiidae, but even between these latter forms and man the differences and difficulties in interpretation are greater than between the highest and lowest of the Old World Apes. There is present in man an extensive cortical area which is small in the Lemurs, New World and lowest Old World forms but which gradually attains a sufficient development so that in the higher Simiidae cortical conditions are reached which can be compared with those in man. This area of cortex, in the lower types, is situated in front of the sulcus lunatus, or rather the bottom of the sulcus, and extends, above, as far forward as the anterior limb of the interparietal sulcus but below it does not encroach to any extent upon the temporal region, only reaching as far forward as the posterior end of the fissura Sylvii. From this strip of cortex with its few and simple foldings as it occurs in the Cebidae and Cercopithecidae, there is developed the major portion of the entire lateral aspect of the hemisphere behind the post-central sulcus. In fact the entire hemisphere, excepting the frontal and the anterior portion of the temporal regions, has been profoundly influenced by the expansion of the area just mentioned, and it will be our task in the following pages to endeavor to trace the course of this great development.

In many of the Prosimiae, in Tarsus and the lowest American forms (the Hapalidae, and even some of the Cebidae such as *Callicebus*), the only sulci present on the lateral aspect of the hemisphere are the Sylvian and some shallow and inconstantly placed furrows which may be considered as interparietal and superior temporal. The lemurs and marmosets are small animals and no great extent of cortex has to be accommodated on the surface of the brain. Yet strangely enough the curious little African

Potto (*Perodicticus*), with a smaller brain than some of the animals just enumerated, has well developed and in a characteristic relative position all the fundamental sulci distinctive of the Primates (fig. 1).

In Potto we meet an undoubted sulcus centralis, although indications of it may be found in certain other Lemurs, both living and extinct. The fissure of Sylvius (*fissura lateralis cerebri*), the superior temporal and interparietal sulci are present in typical form although the last named furrow is shorter and straighter than the corresponding fissure in the apes, of which it represents only the anterior part. This is evident from the drawing (fig. 1), showing the localization, and will be indicated in the discussion which is to follow. Behind the interparietal sulci and quite symmetrically placed are two small transverse fissures, the *ss. lunati*. In addition there is to be seen in each hemisphere a short obliquely placed groove, above and between the Sylvian and superior temporal, at the junction of the temporal and parietal regions and hence may be called temporo-parietal. It lies in the region where areas 7, 21 and 22 (cf. also figs. 12, 13, 14) are, at this early stage, more or less indistinguishably fused (cf. Brodmann 06, fig. 98, Lemur). The correctness of the identification of these fissures in Potto as the lunate and central sulci has been established by examination of the structure of the cortex (unpublished notes of Elliot Smith) and also in the case of the central sulci by the results of C. and O. Vogt's determination of the motor area by electrical stimulation. The arrangement of the rest of the furrows resembles so exactly that found in Lemur that the results of the precise localization of the cortex in that form may be confidently applied to *Perodicticus*. This results in the arrangement shown in figure 1. The pattern of sulci thus revealed is almost identical with that seen in *Pitheca* (fig. 2) one of the more primitive South American monkeys in which the relations of the interparietal and lunate form a connecting link between the Prosimiae and the Apes.

For our purpose, which is to study mainly the transition from the simian to the human condition and the earlier phases of the process which led up to this, it is not directly useful to discuss the

specialised conditions found in *Chrysothrix*, *Mycetes*, *Ateles* and others of those platyrrhine Apes in which a great development of the cortical area controlling the prehensile tail has led to a special-

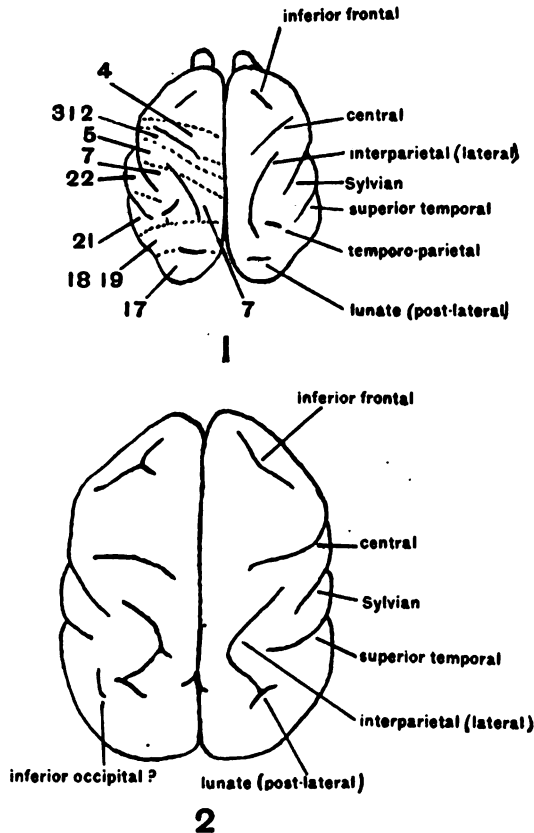


Fig. 1 Dorsal view of brain of *Perodicticus potto*. From a specimen obtained fresh from the Cairo Zoological Gardens. The terms in parentheses are those usually applied to Lemurs. The areas on the left side, with their numbers, are taken from *Lemur* after Brodmann.

Fig. 2 Dorsal view of brain of *Pithecia*. From Elliot Smith ('00), figure 61.

ization of the cortex away from the lines of development which have culminated in the human condition.

As types of a simple and primitive pattern we may refer to *Perodicticus* and *Pithecia* in which the essentials of the fissural

arrangement are shown in figures 1 and 2. From the condition which is common to both the Lemur and the lower Platyrrhine there is an easy transition both to *Cebus* and the Cercopithecidae (figs. 3-7.).

In the members of the latter family the same essential pattern is found; in *Cercopithecus*, *Macacus*, *Cynocephalus* and *Semnopithecus*. The first form has been the subject of a very careful cyto-architectural study by Brodmann ('04) and both Cercopithe-

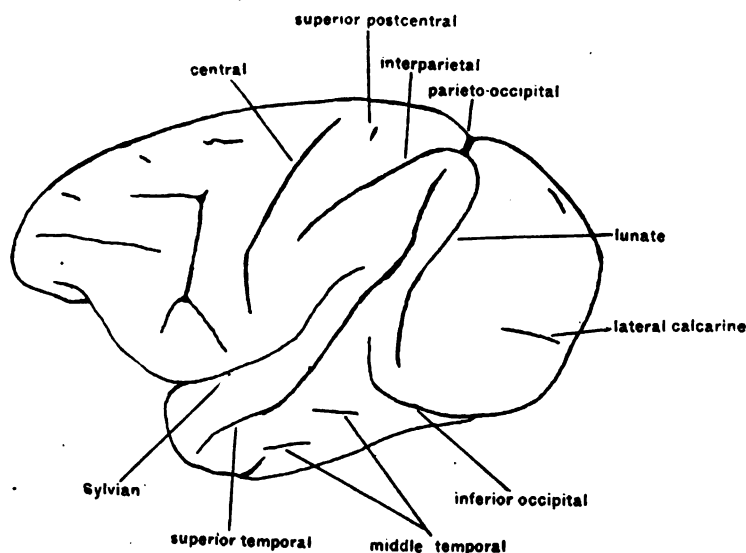


Fig. 3 Left hemisphere of *Cercopithecus ruber*. The sulci unlabelled in the following figures can be identified from this. The parieto-occipital is, in reality, beneath the mesial extremity of the lunate; cf. *Cynocephalus*, figure 5.

cus and *Macacus* by Mauss ('08) from the myelo-architectural point of view, while Schuster, following much the same principle as Brodmann, has investigated the conditions in *Papio* (*Cynocephalus*) *hamadryas*. Of the New World Apes, *Cebus* presents conditions very similar to those in *Cercopithecus* but with a much wider range of variation.

In most of the above mentioned forms the sulcus centralis is usually a quite regularly curved, deep furrow but there is a tendency to irregularity in *Cynocephalus*.

The fissura Sylvii presents some interesting variations. In *Cebus* it is superficially confluent with the superior temporal, whereas in the lower Old World Apes this or more advanced conditions, in which these two fissures are separated, may be found. In *Cercopithecus* and in *Cercocebus* the slightly submerged annectant gyrus between the fissura Sylvii and the superior temporal may become superficial on one or both sides, thus separating more or less widely the two sulci, with the possibility of this separation being more marked on the left side. In *Macacus* the separation of the two sulci is more common; it is still wider and more frequent in *Cynocephalus* and quite constant in *Semnopithecus*. In both these latter forms the Sylvian fissure has become more horizontal, and there may be a slight branching of its posterior end, its course is in addition less regular, on account of the appearance of small transverse temporal gyri, and it may in addition receive a posterior subcentral.

The sulcus temporalis superior (parallel sulcus) is quite characteristic in all cases, as a long deep furrow extending from near the tip of the temporal pole almost to the interparietal sulcus. Its relations to the Sylvian fissure have been noted. With the separation of these sulci, the superior temporal sulcus also tends to become more horizontal, describing more of a curve around the Sylvian and its posterior end may be branched or directed forward. When unbranching and continuing its original course, there may be found, immediately in front of it, above the end of the Sylvian, a small shallow furrow which is compensatory in character. Both the branching of the superior temporal and the compensatory furrow may be found together or more rarely a similar furrow appears behind, just in front of the sulcus lunatus.

The sulcus interparietalis is of special interest and importance since it is situated in a region whose great development is a characteristic feature of the human brain. Its simplest form is seen in *Perodicticus* and its extension backward and outward to reach the lunate in *Pithecia* is shown in figure 2. In its curved form in *Pithecia* it is very similar to the type now under discussion. In *Cercopithecus*, where it is still quite simple, it appears as a very

deep curved sulcus, with a longer anterior ascending limb, the so-called sulcus postcentralis inferior, and a shorter posterior descending limb bent sharply around the upper end of the parallel sulcus. This posterior limb is for the most part submerged in the sulcus lunatus (Affenspalte) while the angle formed by the two parts of the sulcus is separated from the fossa parieto-occipitalis by the first annectant gyrus. One can, as will be seen later, distinguish a very short intermediate portion and these three portions show considerable variation which in its turn depends on a number of factors. In *Cebus* the anterior limb, known as the

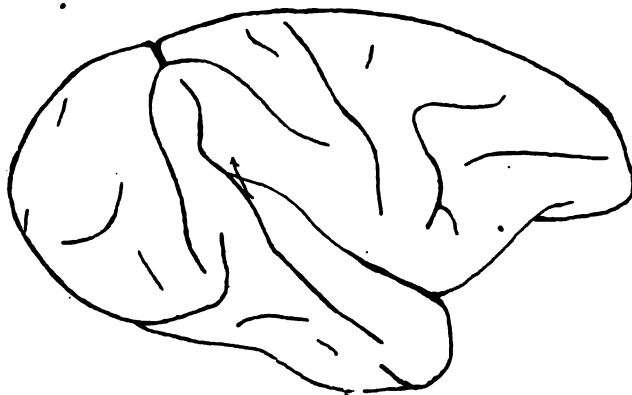


Fig. 4 Right hemisphere of same brain as figure 3. The arrow indicates an annectant gyrus.

inferior postcentral, is quite short and any indications of a superior postcentral or a posterior subcentral are very faint or entirely wanting. In *Macacus* and *Cercopithecus* both are often present or both may be absent or only a superior postcentral is present. *Cynocephalus* and *Semnopithecus* both possess as a rule a superior postcentral and a posterior subcentral and the former sulcus, particularly in *Cynocephalus*, may occasionally unite with the interparietal. The lower end of the inferior postcentral may either turn forward or be branched. At the same time the inferior postcentral has not only approached the central sulcus but has become more nearly parallel with it, and the angle formed

by the inferior postcentral with the intermediate portion of the interparietal is becoming more sharply defined. These changes are due in large measure to the expansion of that cortical area which is situated within the concavity of the interparietal and comprises for the most part area 7. The union of the superior postcentral (so-called) with the interparietal marks off what seems to be, but is not in reality, the gyrus postcentralis of human anatomy. More than one furrow, i. e., the superior postcentral, above the interparietal is rare in forms below the Simiidae (Cunningham '92).

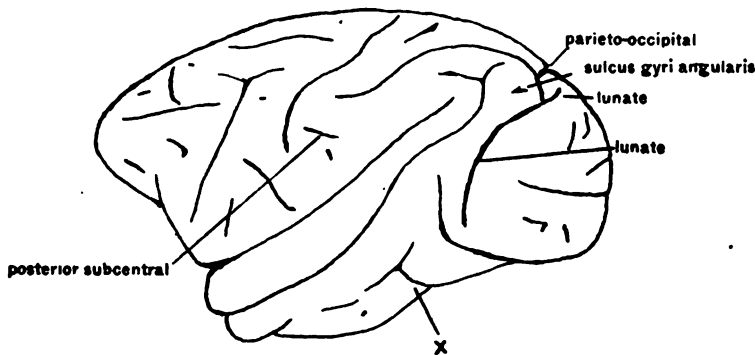


Fig. 5 Left hemisphere of *Cynocephalus*. X indicates the Quersfurche of Zuckerkandl; same brain as figures 6 and 7.

If the interparietal sulcus be carefully examined in *Cercopithecus* or *Cynocephalus*, two important branches will be found which cut into what we may call the superior parietal lobule. One of these, the more constant and well defined of the two, appears as a direct continuation of the sulcus, coming off from its highest point and cutting deeply into the anterior limb of the first annectant gyrus (marked x in fig. 8, *Hylobates*). No evidence whatever of the presence of this branch can be seen on the surface (not figured) since it is very deeply situated, being continued, as it were, from the deepest part, the floor, of the interparietal. The fact that it is deeply placed would corroborate our view that the branch in question presents an integral part of the interparietal, its equal

in all ways, since it represents the posterior extremity of the primitive sulcus which we see in its earliest form in what is also known as the sulcus lateralis in the Lemuroidea (cf. *Perodicticus*). Beyond the point where this branch is joined to the interparietal, this latter sulcus or even the sulcus lateralis is of a newer formation; it is less constant and in the forms we are considering, the Cercopithecidae, it is distinctly shallower, so much so in fact, that one could even speak of an annectant gyrus in many cases. The branch just referred to is to be seen in the left hemisphere of *Pithecia* (fig. 2). On account of the great growth disturbances

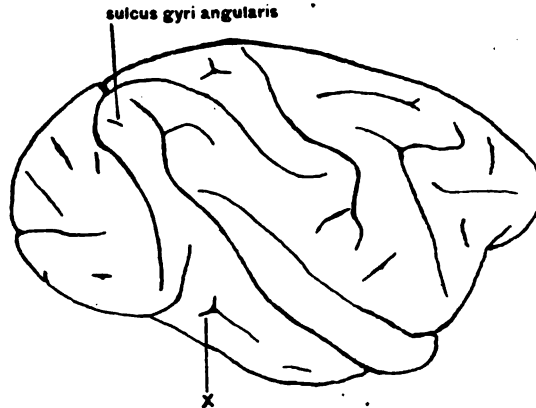


Fig. 6 Right hemisphere of *Cynocephalus*; cf. figures 5 and 7.

occurring in this region, considerable distortion of the primitive conditions in *Pithecia* is inevitable, and the depth to which this branch of the interparietal is buried will be determined in part by the degree to which the first annectant gyrus is submerged. It may be present, but this is doubtful, on the right side of the example of *Pithecia* figured. This dorsal branch of the interparietal sulcus is an important furrow and will be encountered again in *Semnopithecus* and *Hylobates*, where, with the reëmergence of the first annectant gyrus, it also rises to the surface. The other branch of the interparietal is less marked and less constant. It is located somewhat anterior to the first mentioned branch, is usually quite

short and often indicated only by a slight indentation in the anterior wall of the interparietal sulcus. It is rather accentuated by the slight angle in the sulcus at this point and the intermediate portion of the interparietal between these two branches, may be quite distinctly marked off from the anterior more ascending portion (i.e., the inferior postcentral). The portion of the interparietal which we have termed intermediate, would seem to represent the major (posterior) portion of the entire sulcus in *Perodicticus* and to be the equivalent of the *ramus horizontalis* (Cunningham) of man, *sulcus interparietalis proprius*. In more advanced types this segment of the interparietal instead of describing a short curve around the superior temporal, gradually becomes somewhat lengthened, tends to lose its sharp curve and become more nearly parallel with the border of the hemisphere. Its length and course are largely influenced by the same factors which determine the condition of the superior temporal immediately below, i.e., the changes in area 7, which agencies, however, are at work above as well as below the sulcus.

The posterior limb of the interparietal, behind the fossa parieto-occipitalis, lies, as a rule, quite concealed beneath the operculum occipitale and can be examined only by raising or removing the operculum. In its simplest form the sulcus, from its highest point, passes outward and backward under cover of the operculum but does not usually reach the bottom of the sulcus lunatus, being separated from it by the second annectant gyrus. Although this arrangement is found in *Cebus* it is in this form that the greatest individual variation occurs. In *Macacus* (Zuckerkandl '03) and in other forms also, the sulcus may turn suddenly upward, limiting the first annectant gyrus behind. The first annectant gyrus is subject to considerable variation in its relations; it may be well defined and quite superficial, or very much depressed so that a wide, open, superficial connection results between the interparietal and parieto-occipital. This may be but the expression of the degree of development of the cortex forming the first annectant gyrus, for the most part area 19; or, if the operculum is less extensive, as a result of a lessened development of the area striata,

area 17, the first annectant gyrus and posterior limb of the interparietal will be found on the surface to a varying extent. Such an arrangement may occur in any of the forms under discussion, but most frequently in *Cebus*, *Cynocephalus* (fig. 7) and *Semnopithecus*. In *Cebus*, the entire extent of interparietal sulcus, even including a sulcus occipitalis transversus, may be exposed on the surface (Cunningham '92, fig. 46, Elliot Smith, '07, fig. 29). In addition there may be found, lateral to the termination of the interparietal, on the anterior sloping wall of the sulcus lunatus

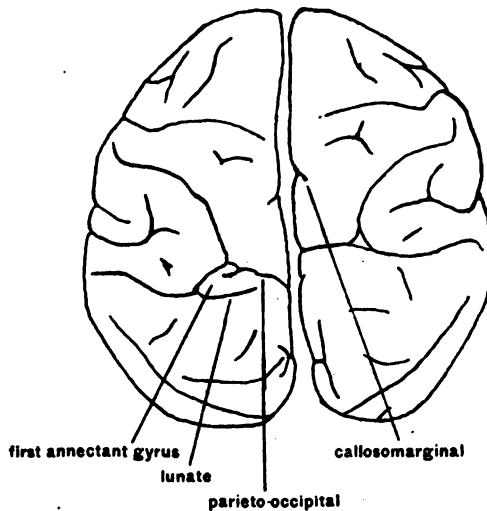


Fig. 7 Brain of *Cynocephalus*, from above and behind; cf. figures 5 and 6.

one or two ill-defined furrows demarcating the second and third annectant gyri, while even a fourth and fifth are figured by Retzius ('06). The last two mentioned are, however, of doubtful significance so far as homology is concerned. A further development of the terminal portion of the interparietal, quite like the conditions occurring in man will be met with later in the Simiidae.

Doubtless the most striking feature exhibited by the brain of the lower Old World Apes is that great deep fissure which traverses almost the entire lateral aspect of the hemisphere, the Affenspalte of Rüdinger, the sulcus lunatus of Elliot Smith. Absent in the

Hapalidae and the lowest of the Cebidae such as *Callithrix* and *Aotus* and inconstant in such forms as *Chrysothrix* and *Pithecia*, it shows its greatest variation in *Cebus*. Here it may be represented by a relatively short shallow furrow which is not unlike its first appearing homologue in the Lemurs, or it may be deeply operculated and located considerably farther forward. In the *Cercopithecidae* it is always well developed. The relation of the free edge of the operculum to the interparietal and parieto-occipital will vary in accordance with the degree of operculation, whereas the bottom of the lunate sulcus is much more constant. The sulcus lunatus, as outlined by the anterior edge of the operculum, begins near the mesial border of the hemisphere, overlapping it in some cases, extends outward and slightly forward and describes a gentle curve whose convexity is directed forward. It stops some little distance above the inferior margin of the hemisphere, owing to the interposition of the inferior occipital sulcus. The relief of the anterior wall has already been mentioned and the posterior fits into this more or less closely. In most cases there are submerged beneath the operculum the parieto-occipital fossa and the termination of the interparietal sulcus and the corresponding first and second annectant gyri, or even additional gyri, which structures will become superficial with the recession of the operculum.

Regarding the phylogeny of the lunate sulcus we may quote from Elliot Smith's (l.c., '08, p. 167) writings on the extinct Primate, *Lemur jullyi*:

There seems to have been in this brain an exceptionally extensive representation of the furrow which in my former memoir I called 'post lateral'—an identification which I have since proved to be absolutely exact, because this furrow, both in the Carnivora (in the brain of which this sulcus was first so called) and in such Prosimiae as *Lemur*, *Nycticebus*, *Perodicticus* and *Propithecus*, forms the cephalic boundary of the visual cortex or area striata. This observation, which has not hitherto been published (except verbally at a meeting of the Anatomical Society on June 1, 1901) has been recently confirmed in the case of *Nycticebus* by Oskar Vogt. I refer to this apparently irrelevant matter here to emphasize the fact that when the sulcus lunatus ('Affenspalte') first makes its appearance in such lowly Cebidae as *Pithecia* it presents the same relationship to the area striata as the sulcus postlateralis exhibits

in the Prosimiae—a relationship which undergoes a progressive modification in the higher Cebidae and the Cercopithecidae as the result of the differentiation of the neopallial areas fringing the area striata and the deepening of the sulcus lunatus to accommodate these expanding stripes of cortex. In other words the sulcus lunatus (postlateralis) of the Prosimiae, while retaining its similitude to and real identity with the postlateral sulcus of the mammals, presents a much nearer approximation to the condition found in the lowlier Cebidae than the latter presents to the state of affairs met with in, say, Cercopithecus.

As we gather from the description of Kohlbrugge, his Affenspalte owes its existence to the sinking in of the first annectant gyrus and has, consequently, as little to do with the Affenspalte (sulcus lunatus) as that term is usually employed (Elliot Smith, Cunningham, Retzius, Brodmann and with certain modifications, Zuckerkandl), as the sulcus in question has to do with the first annectant gyrus.

The sulcus occipitalis inferior (occipitalis lateralis of Retzius, occipito-temporalis lateralis of Brodmann) is always very well marked. Its first beginnings seem to be rather indefinite but we are inclined to look for them in the small groove figured in the left hemisphere of Pithecia just lateral to the lunate. Typically it begins in advance of, and above the lower end of the lunate, passes downward and backward bending around the lunate and extends for a variable distance toward the occipital pole. That portion behind the lunate is the more variable, it may be entirely on the lateral aspect of the hemisphere, approximately parallel with the margin or its course may be more oblique carrying it onto the tentorial surface. Its relations to the lunate are quite constant and it will be found nearer or farther from the margin depending upon the downward extent of the lunate. Occasionally its anterior part, in front of the lunate, may be separated and the remainder of it joined to the middle temporal. Its posterior extremity may also be bent upward so that the sulcus describes a sharper curve around the lunate. As a rule it is quite deep, even as deep as the lunate and its superior lip is operculated. As will be seen later, the sulcus occipitalis of the Simiidae and of man cannot be derived from the sulcus just described. It is evident therefore that a new term is required and in referring to that

sulcus in the simian brain which is the real derivative of the inferior occipital of the Cercopithecidae I shall use the term sub-lunate which name might be applied with equal propriety to the inferior occipital of lower forms and the last mentioned term in turn be reserved for the newer fissure of the Simiidae.

The sulcus temporalis medius is represented by a varying arrangement of short shallow furrows, running in various directions, below, but in general parallel with the superior temporal. One of these furrows we shall meet again since it assumes considerable importance.

The large expanse of cortex situated behind the sulcus lunatus may be quite free from any furrows as not infrequently happens in *Cebus*, *Macacus* and *Cercopithecus* or only one or two slight grooves may be found. In the majority of all forms however, there is seen a distinct but not deeply incised furrow, extending from the occipital pole upward toward the center of the lunate. It is the external calcarine fissure of Cunningham, sulcus occipitalis of Retzius, sulcus opercularis of Brodmann. In conformity with the B.N.A. usage it may be called the sulcus calcarinus lateralis. Its length and depth vary although its general direction and location are quite constant. In addition smaller much shallower accessory furrows are frequently present, above or below the lateral calcarine or the latter may be branched. The retrocalcarine sulcus usually ends in a very pronounced vertical bifurcation on the mesial surface but occasionally it may remain unbranched and extend outward upon the occipital pole above or between the branches of the lateral calcarine.

The sulcus praelunatus, which is the sulcus occipitalis lateralis of Eberstaller, so frequent in the Simiidae, is only slightly or occasionally indicated (cf. Retzius '06; *Nasalis larvatus*, plate 33, *Macacus*, plates 24 and 27).

In addition to the above mentioned sulci, small shallow inconstant foldings of the cortex may occur in front of the sulcus lunatus, between it and the superior temporal and to these we shall refer later.

From the foregoing account it will be evident that the lower Old World forms conform quite closely to a rather simple plan in

the general arrangement of the sulci under consideration, the nearest approach to the Gibbons being found in *Semnopithecus*.

Passing to the lowest of the Simiidae, *Hylobates* (figs. 8-10), a distinct advance and increase in complexity are at once noted, and indeed the conditions in the Gibbon are essentially those found in the other Simiidae, Chimpanzee, Orang and Gorilla. The following account of the morphological arrangement in *Hylobates* may be taken as representing the general pattern on which also the corresponding area in higher forms and in man has been modelled. The increased development of certain cortical regions has placed the brain of the Anthropoids above that of all the other Apes and similar lines of progress have raised above all the brain of man.

The fissura Sylvii has taken here a rather more horizontal position and at the same time its length has decreased; possibly it may be slightly shorter on the left side. Its posterior end is variable: it may lie directly in the continuation of the rest of the fissure, but more commonly shows some tendency to branch, often into a typical arrangement of ascending and descending limbs, or it may turn upward for a short distance. A posterior subcentral is not infrequently present and may unite with the Sylvian. Indications of transverse temporal gyri are often found.

The sulcus temporalis superior is still a long and uniformly deep furrow. It gradually approaches the Sylvian and sweeps round it, in a very distinct curve, upward or even forward. Its course may be somewhat irregular from the presence of interlocking gyri in its walls, particularly just before it turns upward, at which point a descending branch is not uncommon. The upturned end of the sulcus is subject to some little variation. It may be branched or not, and its location in the inferior parietal lobule may vary. Both these conditions and the presence of the smaller sulci in this region are often compensatory in character and closely associated with, and due to, changes taking place in this area.

The sulcus centralis is, as far as we are concerned, unchanged.

The sulcus temporalis medius is present in the form of a few irregular furrows disposed in general parallel with the superior sulcus and of which the posterior furrows show a greater tendency

to assume a transverse position. The most posterior and at the same time the best developed of these cross furrows may unite at times with the inferior occipital. This is Zuckerkandl's Querfurche and is, as we shall see later, an important furrow.

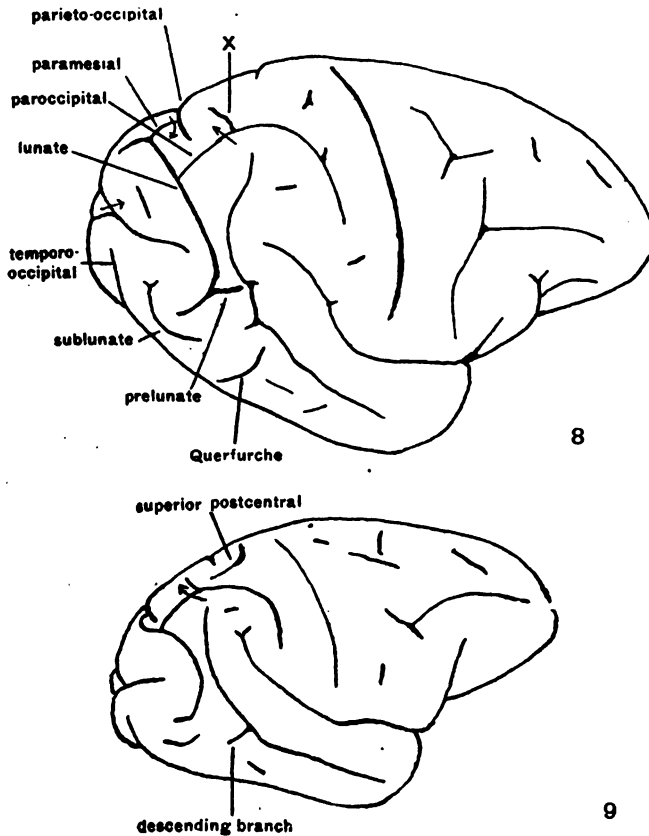


Fig. 8 Right hemisphere of Hylobates Mülleri. X indicates the dorsal branch of the interparietal discussed in the text.

Fig. 9 Right hemisphere of Hylobates Mülleri.

The interparietal sulcus presents a more advanced condition than in *Semnopithecus*. The ramus occipitalis is usually quite distinct on account of the increase in size, and the emergence of the first annectant gyrus. The continuation of the ascending

part of the interparietal, previously described in *Cercopithecus*, is still present and now on the surface but otherwise sustaining the same relations as before. It is significant that in certain cases this part of the interparietal may be separate from the rest and then the furrow limiting the first annectant gyrus in front is connected with the anterior part of the interparietal above. This limiting furrow of the annectant gyrus varies somewhat and with it the size of the gyrus, being found at times very far forward. It is further markedly influenced by the direction and depth of the fossa parieto-occipitalis.

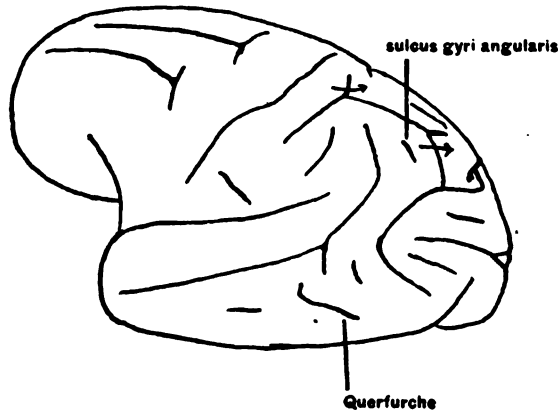


Fig. 10 Left hemisphere of *Hylobates Mülleri*.

The superior postcentral fissure is distinctly variable or may even be absent. It is sometimes found in a very well defined form uniting with, but much shallower than, the inferior, or between the two there may be an annectant gyrus. When this occurs we have, sharply defined, for the first time all the classical rami of the interparietal. This superior postcentral, lying between areas 2 and 5, is not the sulcus having the same name in *Cercopithecus* since in the latter it lies between areas 1 and 2. The point is perhaps a fine one. There is often an inverse proportion in the relative development of this sulcus and the posterior extremity of the calloso-marginal, compensatory in nature. Occasionally the first annectant gyrus may be so large that its anterior limiting fur-

row is found very far forward simulating a superior postcentral. Indications of a superior parietal sulcus are sometimes met with.

The lunate sulcus is quite characteristic in that its mesial extremity has been pushed back by the large first annectant gyrus. This end of the lunate often turns abruptly forward so that it might be confounded with the paramesial which is at times indicated, but the relations here are often difficult to interpret. The occipital ramus usually turns slightly upward under the operculum and is unbranched. Just above this last named sulcus,

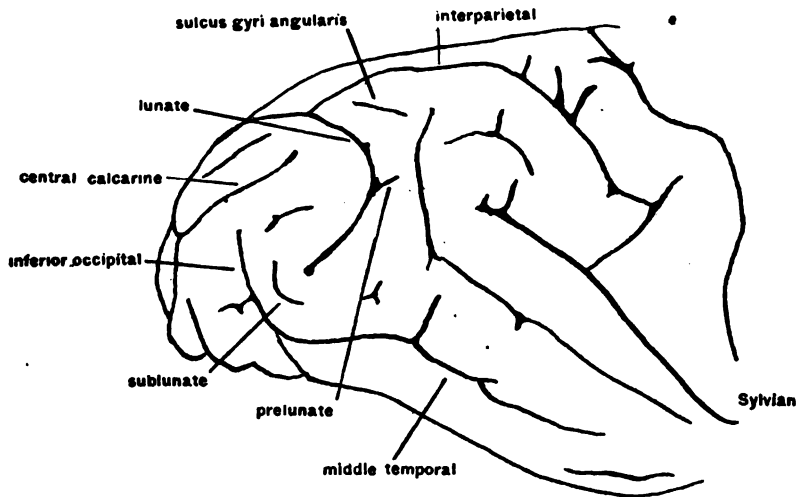


Fig. 11 Posterior half of right hemisphere of Gorilla. From a photograph of a specimen in the Royal College of Surgeons, London.

there may appear on the anterior wall of the lunate a short sagittally placed furrow which may reach the surface and the significance of which is doubtful. It might accommodate a slight cortical increase which is cared for in other cases by the "new branch" of Cunningham which will be noted later. A deep short prelunate, continued backward into the lower end of the lunate is commonly found and is separated from the parallel sulcus by a more or less deeply placed annectant gyrus. Certain other sulci will be considered in what follows.

Although fashioned on the same general plan as found in the Gibbons, there is in all the other Anthropoids a very great increase in complexity and variability of detail. Among the essential differences, advances, may be mentioned the following points, a general discussion of which will be entered into later (fig. 11).

The fissura Sylvii is more horizontal and relatively shorter, its borders are much indented, either from the transverse sulci of the opercula or from its union with neighboring sulci; its posterior end is in particular variously branched and either separate or united to the sulci of the supermarginal gyrus.

The parallel sulcus still preserves in a measure what we may call its primitive form in that it extends from the temporal pole almost to the interparietal. It has suffered, however to some extent in that its course is less regular, there being frequent indentations of its walls and interlocking gyri. At its posterior extremity it is usually markedly branched and irregular and as it turns around the Sylvian it very commonly has a deep branch directed downward and backward, a ramus descendens. Its posterior third may be quite independent, as a sulcus angularis.

There have been no very radical changes in the second temporal sulcus, but there is the tendency for it to form a continuous furrow. Its posterior end may be in relation with a Querfurche, confluent with the inferior occipital or independent, sometimes turning abruptly upward.

Although the sulcus lunatus is still a conspicuous feature, it lacks the regularity and constancy of form and position which it exhibits in the lower primates. Relatively smaller, it is also placed nearer the occipital pole and the mesial extremity has retreated more than in the lateral, especially in Gorilla and Orang, so that viewed from behind its direction approaches the horizontal and a portion at least of the posterior limb of the first annectant gyrus is exposed. The prelunate is much like that in Gibbon.

A paramesial sulcus may be present, especially in Gorilla, on account of the retraction of the mesial end of the lunate.

As regards the interparietal system, the conditions in the Orang and Gorilla are most like those in the Gibbon, while in the Chim-

panzee there seems to be a wider range of variation and the ramus occipitalis is more completely covered by the operculum. The postcentral sulci show considerable changes, are more branched and irregular, the superior is often separated and may unite with a superior parietal. The ramus horizontalis is much more irregular on account of ventral and dorsal offshoots which show a certain compensatory relationship to the terminal branches of the Sylvian and particularly the parallel sulcus. Any of the conditions found in the Gibbon may occur, but with the natural tendency for the new furrows to increase in number; they are placed in general more or less parallel with the superior temporal or in a radiating manner perpendicular to the interparietal, they may unite with any neighboring furrows or be independent. The dorsal branches of the interparietal are especially variable and irregular.

In the great majority of brains the ramus occipitalis disappears beneath the operculum. This part of the parietal lobe which is covered by the operculum, namely the anterior sloping wall of the lunate sulcus, is an important area, taken up in large part by the three or even four and five external annectant gyri, out of which is completed (Zuckerkandl) the parietal lobe. As previously stated, the extent of the operculum will determine the degree to which these gyri are submerged, and its recession will bring them to the surface in their natural order from within outward. The first annectant gyrus of Gratiolet, or arcus parieto-occipitalis of Eberstaller is looped around the fossa parieto-occipitalis as the latter cuts the margin of the hemisphere. It is bounded laterally by the ramus occipitalis and its size is largely dependent upon the depth of the fossa. It is the first of the annectant gyri to make its appearance on the surface and indeed its anterior limb is always uncovered. The second annectant gyrus usually forms a loop around the termination of the occipital ramus, separating it from the bottom of the lunate sulcus, while the remaining annectant gyri are small variable sagittal foldings, the fate and importance of which are much more problematical. In the Chimpanzee and Orang there is frequently given off under the operculum a branch which passes upward and backward

behind the first annectant gyrus. This is the new branch of Cunningham (l. c., '92, p. 225) and the occipital ramus has now the appearance of ending with a terminal bifurcation, of which the two limbs are quite different morphological value since the lateral limb is a continuation of the original fissure and is, as we shall see, alone of significance as a limiting fissure.

The sulcus praelunatus, occipitalis lateralis, is common and may be conceived as arising from one of the furrows which separate the more lateral annectant gyri, possibly between the third and fourth, the extreme development of one of these gyri might give rise to a gyrus translunatus.

All that remains of the inferior occipital of the Cercopithecidae is a small shallow furrow or a few fragments, which, as above stated, we shall refer to as the sulcus sublunatus. It may be entirely wanting or again fairly well developed as it is in the Gibbon. The sulcus occipitalis inferior of the Simiidae and of man presents a much more difficult problem: it might be brought into relation with the Querfurche of Zuckerkandl or with fissures in the continuation of, or representing the descending branch of, the superior temporal. Three principal varieties are found, in which it is dependent or confluent in front with the middle temporal or with the temporo-occipital (inferior temporal). These relations as also the position of its posterior extremity, which may be found on the lateral or tentorial aspect of the hemisphere, are intimately associated with the position of the lunate and its associated areas. In a brain in which the lunate stops some distance above the margin of the hemisphere the inferior occipital sulcus will be on the lateral surface and often united with the middle temporal, while in the opposite condition it is on the tentorial surface and continuous with the temporo-occipital, but in any case its posterior end may reach the tentorial surface.

Before we proceed farther in our discussion it will be necessary to define what we mean by the sulcus lunatus, for it is with the region immediately in front of this sulcus that we are especially concerned. Any definition which involves homology, as does this one, must be the most comprehensive possible without being in any wise indefinite or equivocal. By comprehensive

we mean that it must be based on such fundamental genetic principles as will render it applicable to the greatest number of cases, will give it the greatest heuristic value without its invalidation as an expression of homology.

To begin with, the term "Affenspalte" is open to objection and the same criticism naturally applies to such designations as the sulcus simiarum of Retzius and the sulcus simialis of Brodmann, for the simple reason that the sulcus is not peculiar to any Ape. This is a point however of minor importance, the sulcus certainly reaches its greatest development in the lower Old World Apes and as names these have much more to recommend them than many of definitions which have been fabricated for them. We would define therefore, the fissure to which we shall hereafter apply the term sulcus lunatus, as that fissure in the primate brain, which, be it operculated or not, and be its relation to other fissures what they may, is situated immediately in front of that portion of the area striata which is located on the lateral aspect of the hemisphere. In the definitely operculated forms of the sulcus, a characteristic and quite distinctive feature is the extension of the area striata almost to the free edge of the operculum and this relation is causally related to the development of the operculum. In proportion as the operculum is ill-developed the less exact becomes the coincidence of the anterior limit of the area striata with the margin of the operculum. We are well aware of the grounds upon which this definition is based, but there can be no doubt that the occipital operculum and with it the sulcus lunatus are directly and genetically related to the development of the area striata and that the relations of these to any neighboring structures are secondary and therefore of secondary importance.

The conception of Kohlbrugge, referred to above, is altogether impossible since it restricts the term to a quite fortuitous combination of furrows which is only found in certain forms and even in them with individual variations. Zuckerkandl's view, which is accepted by Sergi, is more acceptable, it nevertheless suffers from his defining the essential in terms of the non-essential and has made, as it were, the sulcus lunatus a function of the ever-varying first, second and third annectant gyri. It seems that

Zuckerlandl attached too much importance to the bottom of the sulcus lunatus and its relations, whereas it is the free border of the operculum that is in most intimate association with the area striata and it is in the relations of this border also that the growth-changes both in this and other areas have their most marked effects. The annectant gyri occupy a peculiar and yet perfectly natural and necessary relation to the lunate sulcus. If the bottom of this sulcus be taken as the dividing line between the parietal and occipital lobes, then it of course follows that any gyri in relation with it will be annectant, i.e., uniting the two lobes and further must be present unless the lunate be the only fissure developed. One could with equal propriety speak of annectant gyri in the gyrencephalic Lemurs where there is neither sulcus lunatus nor operculum. In *Nycticebus tardigradus*, the presence of a lunate sulcus is proved, according to Brodmann ('07, figs. 42-43 and p. 332), by its relation to the area striata, but in this brain annectant gyri, as the term is generally employed, are entirely wanting. Doubtless many of Zuckerlandl's sulci lunati, or rather "Affenspaltenreste," would fall under our conception of that sulcus, but for other reasons than those upon which he has identified them.

It would be quite hopeless to seek in man or even in many lower primates for a lunate sulcus answering to the condition found, e.g., in *Cercopithecus*. Neither does it appear correct to seek to homologize the sulcus in man with only a part of the sulcus in the Apes. It is an entirely indefensible restriction to demand that the relatively greatly reduced lateral portion of the area striata in man should be bounded in front by a furrow, the sulcus lunatus, presenting exactly the same relations as in *Cercopithecus* where the relative cortical development in front and behind the sulcus is exactly the reverse of what it is in man. According to Brodmann ('08) the area striata may constitute 10 per cent of the entire cerebral cortex in many apes and this relation may rise to almost 15 per cent in the Lemurs, while in man it has dwindled to only about 2 per cent. (For other interesting comparisons of homologous areas consult Brodmann ('09) and Henneberg.)

Given two homologous neopallial areas, e.g., the area striata in two forms, it would follow that their bounding sulci, called into being by the increasing development of these very areas, would be homologous and if the entire areas are homologous then the entire extent of the sulcus of the one would be comparable to the entire sulcus of the other, and while the sulcus may be interrupted—gyrus translunatus—or disrupted, one should not institute a comparison between the parts of one and the whole of the other. If anyone is in doubt about the homology of the sulcus lunatus let him compare Brodmann's ('06) figures 14, 26, 46 and 76 which show unmistakably the relation of the striate area, operculum and lunate sulcus in *Homo*, *Simia satyrus*, *Semnopithecus* and *Cercopithecus*. Many examples of the sulcus lunatus in man might be culled from the literature (cf. Retzius, '96) where they have often been passed unnoticed or have received other, frequently inappropriate designations. It is natural to suppose that the foldings of the cortex, the gyri and sulci, incident upon its continued development and increase in volume and area, should exhibit some intimate relation to those areas, the development of which, often very unequal in degree, has determined their formation.

But we are not reduced to the extremity of depending upon *a priori* grounds. The extensive researches of Elliot Smith, Campbell Brodmann, Mauss and others have placed this relation beyond any reasonable doubt. Although the last two writers might disclaim any intention of putting such an interpretation upon their findings we cannot do otherwise than look upon them in this light. If there seems to be no exact and perfectly constant relation between areas and surface markings, we have only to remember that in very many cases the precise limits between different areas have not yet been drawn. On the contrary, one cannot expect that the thirty different areas mapped out by O. Vogt in his *isocortex parietalis*, especially the numerous smaller areas in the region of the central and postcentral sulci, will or can be all in the same relation to sulci and gyri, for many of them, as compared with the areas of Brodmann and Mauss, are quite small and it is

well known that there exists a relation between the thickness of the gray lamina and the size of its foldings—cf. the cerebellar cortex and the olive. But within these limitations his diagrams show a considerable correspondence between areas and gyri. Furthermore, with two hereditary factors at work, in the first place the extent of the cortical area, with its individual variations, and secondly its associated foldings, we can look for no more than a general, though none the less essential, correspondence and this can only be determined by the investigation of a large number of cases. These considerations are amply sufficient to explain the findings of Brodmann, whose descriptions are those of individual and probably, though not necessarily, of average conditions. It is very interesting in this connection to note that, in that area which is most sharply marked off from others the relations of the associated furrows are most typical and constant. We refer to the area striata, the so-called visual cortex.

We may take occasion here to mention the various kinds of sulci which may arise in connection with the development of a given area and illustrate them on the area striata. They may be within a given area or between adjacent areas or may sustain a similar relation to more than one area. These inter- and intra-areal furrows may be further grouped in the following manner (Elliot Smith '07, p. 202). Axial furrows are formed by folding within an area; the bounding furrows are either sulci limitantes or sulci operculati depending upon the correspondence of the limits of the area with the bottom of the sulcus or with one lip (operculum) of the same. The sulcus retrocalcarinus or posterior calcarine is an axial furrow, the sulcus calcarinus proprius, anterior calcarine is a sulcus limitans, while the sulcus lunatus, and typically also the inferior occipital in lower forms are sulci operculati.

Some general conclusions on the localization in primates may be drawn from the works and certain brain-charts reproduced here (figs. 12–17) of the above cited investigators. It is evident that the increase in the complexity of fissure formation and in the number of areas which can be distinguished has not been uniform. This lack of uniformity is, of course, the very essence of specialization. In other words, there has been relative increase or decrease in

many regions with the introduction of a gradually increasing number of newer areas derived from the further differentiation and subdivision of older ones. The cortical areas which have suffered the most relative reduction are those in relation to the special senses, although there is no intention to refer here to that classical example of cerebral reduction, the rhinencephalon. The motor and general sense areas, on the contrary, are remarkably

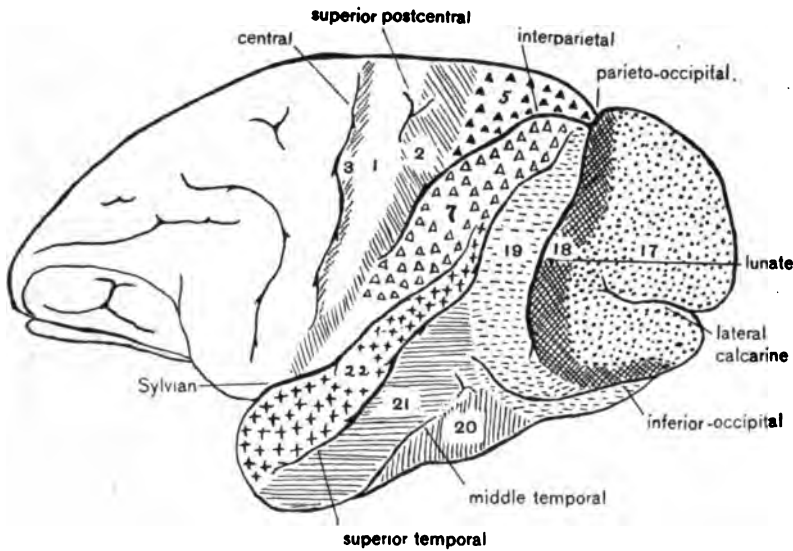


Fig. 12 Cortical areas in *Cercopithecus fuliginosus*, after Mauss ('08), figure 2. In figures 12 to 17 inclusive, the same signs are used to denote the various areas, but certain changes have been introduced in the signs used. For further explanation see text.

constant in their extent and fissural relations. It is further clear that two very striking changes have taken place. Firstly, a very marked reduction in the extent of the area striata, type 17 of Brodmann and Mauss, especially as regards its extension onto the lateral aspect of the hemisphere, and a relative increase, as compared with the area striata, of the parastriate and peristriate areas (Elliot Smith), occipital and preoccipital areas or types 18 and 19 of Brodmann and Mauss. Secondly there have become

differentiated those areas which make up in large part the superior and inferior parietal lobules and a portion of the temporal lobe. These newer areas, the marked development of which is so characteristic of the human cerebrum, correspond in a general way with the association areas of Flechsig, the so-called silent areas. A comparison of the brain of the lower Primates with that of man will show that what may be referred to as the parietal, occipital and temporal sense areas, regions subserving essentially sensory functions, make up the bulk of this part of the hemisphere, while in man they are widely separated by the differentiation and intercalation of newer and higher areas. Following out the comparison that has been made in describing man as a "Hirnwesen" and all lower animals as "Darmwesen," one is tempted to ascribe to these forms the possession of a mind-brain and a sense-brain respectively.

Without venturing any assertions as to the factors concerned in the production of microcephaly, it may be noted that these brains often present a number of pithecoïd characters, depending in part upon the degree of non-development. Some of these are to be found in the region of the Sylvian fissure and the insula. The central fissure is not so deep as the interparietal which has a tendency to become simplified. A sulcus lunatus is common and often operculated. The retrocentral portion of the cerebrum has suffered most on account of the great reduction in the occipital and parietal regions. An examination of the drawings of the two microcephalic brains described by Cunningham ('95) shows that the parietal and occipital regions, made up of areas 5, 7, 39 and 40 and areas 17, 18 and 19 respectively, are very diminutive. The parietal region is so small that the sulcus lunatus is well forward, just behind the parieto-occipital, while the development of the cortex behind, although forming a sulcus lunatus, has not been sufficient, in either of these brains, to operculate that sulcus. The weight of the smaller brain while fresh was 352.5 grams, its relation to the body weight being 1:110 while in size it might easily be surpassed by the brain of the anthropoids or even by that of the lower apes. The main defect is in area 7 and its deriv-

atives, areas 39 and 40, and the mental status of these individuals is quite in harmony with the general results of anatomical investigation.

In *Cercopithecus* and indeed in *Hylobates* and *Simia* also, the regio parietalis (areas 5 and 7 and the derivatives of the latter, 39 and 40) is quite small as compared with the regio occipitalis (areas 17, 18 and 19 and the derivatives of the last) and is represented by a narrow band of cortex interposed between the occipital and temporal regions behind and the postcentral region in front. In the discussion which follows we shall have occasion to make use of the findings of Brodmann, Mauss and others and if we seem to allow ourselves some latitude in the application of these findings it is to bring them into line with other researches and for the additional reason that they represent only single, isolated cases in which the rôle played by individual variations in the relative extent of various areas and in the fissural pattern is difficult or impossible to determine and hence these charts cannot claim the significance they might, were they based on the examination of a large series of brains. The diagrams of Brodmann ('08) representing the various areas in *Cercopithecus* and in *Homo* and those of Mauss ('11) in *Cercopithecus*, *Hylobates* and *Simia*, although based on different principles, present a striking correspondence and will be of great value for the understanding of what is to follow. Two differences between these writers may be noted as regards the brain of *Cercopithecus*; while the combined areas 18 and 19 occupy about the same extent of cortex, the area 18 is in Brodmann's charts much more extensive than those of Mauss. But a much more important discrepancy is found in the parietal region. According to Mauss the area praeparietalis, type 5, makes up the entire region above the interparietal sulcus and the area parietalis, type 7, does not extend beyond that fissure. Brodmann, on the contrary, finds type 7 extending upward beyond the interparietal, between 5 and 19, onto the mesial aspect of the hemisphere. On this point we shall follow Brodmann, since he finds a corresponding interposition of area 7 between 5 and 19 in *Hapale* and *Lemur* and even in *Carnivora* (*Cercoptes*).

Schuster gives a similar distribution for his inferior parietal type in the baboon, *Papio hamadryas* (*Cynocephalus hamadryas*). It may be also mentioned at this time that the more constant relation between areas and fissures is to be found in the work of Mauss.

As indicated earlier in this paper, we are more especially concerned with the area praeparietalis, type 5, the area parietalis, type 7 and their associated furrows, and the area praeoccipitalis,

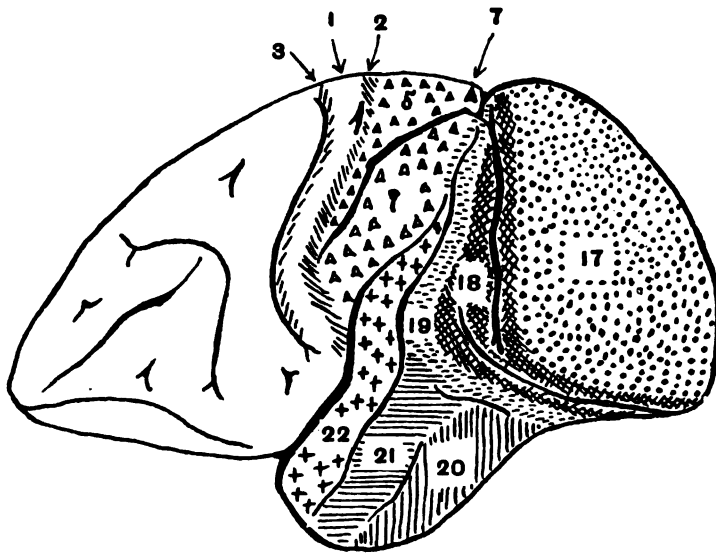


Fig. 13 Cortical areas in *Cercopithecus*, after Brodmann ('08), figure 90.

peristriata, type 19. For our purpose it is unfortunate that the Gibbon, brain figured by Mauss does not exhibit a more typical arrangement of gyri and sulci. A comparison of *Hylobates* and *Cercopithecus* (figs. 12-14) will make it plain that the greatest change has taken place in the area parietalis, type 7. Present to a small extent above the interparietal (Brodmann) in *Cercopithecus*, it forms a very extensive area above that fissure in *Hylobates*. The preparietal area, 5, has taken up what seems to be a new position, being drawn out as a long narrow zone between the area parietalis and the area postcentralis caudalis, type 2,

lying in front of the inferior postcentral below, but behind the so-called superior postcentral above while an extension of 7 seems to have taken up the position above the interparietal formerly occupied by 5. It would appear, therefore, that the fissure which is apparently so similar in the lower apes and the Simiidae and known, in its entirety, as the interparietal, exhibits certain differences in the two groups in that the various segments comprising the sulcus differ relatively in the degree of their development. The

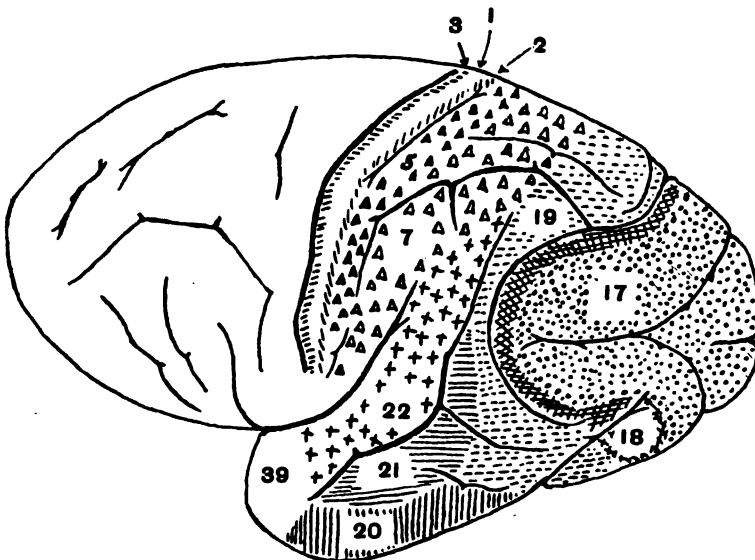


Fig. 14 Cortical areas in *Hylobates*, after Mauss ('11). Certain small areas are not shown.

sulcus postcentralis superior which is less constant in the lower apes can hardly be looked upon as the same sulcus in higher forms. While Mauss does not in his charts of *Hylobates* and *Simia* subdivide area 7, he states in the text that it is separated into an upper and a lower portion, without however, indicating where the dividing line would fall. Doubtless, as we shall see later, it would correspond with the interparietal. It is often difficult or actually impossible from the charts and sections of these writers to determine the exact relation between many areas and fissures. To

fit certain sections, of which there are rather too few, satisfactorily into their chart is quite hopeless, and when one is dealing with other individual brains for the sake of comparison the difficulty is still more pronounced.

As an example of one of these disconcerting discrepancies, it may be mentioned that Mauss ('11) in his text (p. 444) states that area 7 is bounded in front by areas 2 and 5 and behind by areas 19 and 22 and indeed this is quite obvious from his chart of the Gibbon (fig. 24), but the section represented in figure 13 shows areas 5 and 19 in direct relation with each other in two places, apparently a considerable extent of contact. The same confusion exists in the description of the brain of the Orang (q.v.) where chart and sections are, in certain respects, quite irreconcilable. To what extent this strip of cortex, deeply buried in the interparietal (a part of area 5 according to the *sections* of Mauss), may be looked upon as a part of the primitive area 7—instead of 5—and in how far a comparison might be instituted between it and the similarly placed “visuo-sensory band” of Elliot Smith (l.c. '07, p. 245), which unites the general sensory and visual cortex, we shall leave for the present an open question.

In *Cercopithecus* the preoccipital, a peristriate area, makes up the larger part of the first annectant gyrus, a varying extent of the posterior limb being composed of occipital or. parastriate cortex, while a smaller part of the anterior limb, extending from the superior parietal lobule to about the point where, as noted earlier, the interparietal is continued upward limiting the first annectant gyrus in front, is occupied by the parietal area (preparietal, Mauss). The preoccipital area extends laterally along both sides of the descending limb of the interparietal, the anterior wall of the sulcus lunatus and the second temporal gyrus. The arrangement of areas 2, 5 and 7 can be seen from the accompanying the diagrams, the anterior wall of the inferior postcentral (interparietal) being formed low down, near the surface by area 2; and more deeply by 5, the latter being more deeply situated than would appear from the charts. As regards areas 18, 19 and 22 there is some difference in the descriptions of Brodmann and Mauss. According to the former, 18 occupies both walls of the

lunate sulcus and extends over the second temporal gyrus and halfway down on the posterior wall of the superior temporal sulcus, while area 19 is confined, at this level to the bottom and to an equal extent to the anterior and posterior walls of the Sylvian. Mauss finds that 18 only extends slightly beyond the bottom of the lunate into its anterior wall and in front of this, 19 forms the second temporal gyrus stopping at the bottom of the superior

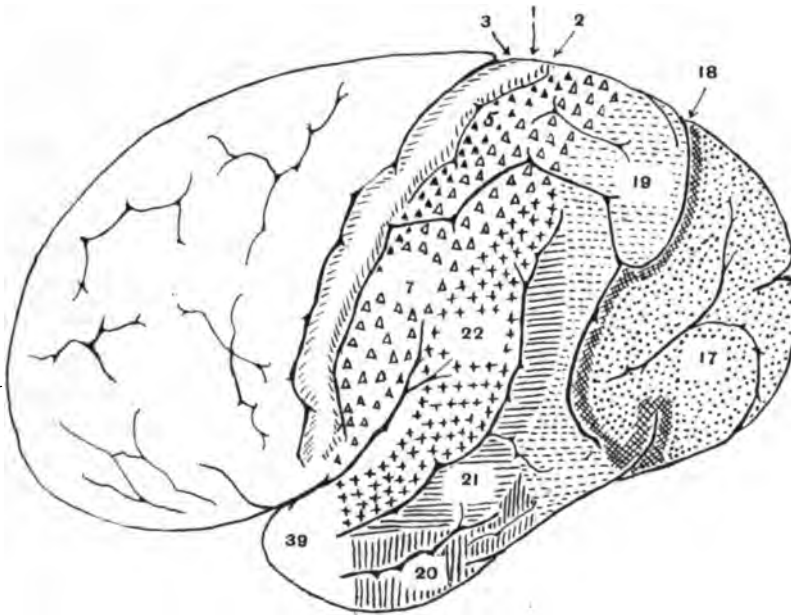


Fig. 15 Cortical areas in Simia, after Mauss ('11): Certain smaller areas have been omitted.

temporal sulcus. Area 19 is not so sharply defined in front as it is behind and shows some inconstancy in its structure. Area 22, superior temporal, constitutes almost the entire first temporal gyrus; it extends more deeply into the Sylvian where it reaches the bottom, than into the superior temporal sulcus.

In the Gibbon the increased development of areas 7 and 19 both above and below the interparietal, with an associated recession of the area striata, 17, has given rise to an increase in size in both the superior and inferior parietal lobules, an increase

in the length of the interparietal sulcus and, on account of the greater reduction of 17 near the mesial border, to the characteristic form of the lunate sulcus. This enlarged parietal area is due to the expansion of area 7, separating the upper and lower parts of which we have the sulcus interparietalis proprius (*ramus horizontalis*). As has been already shown, this part of the interparietal is very short in all forms below the Simiidae, forming

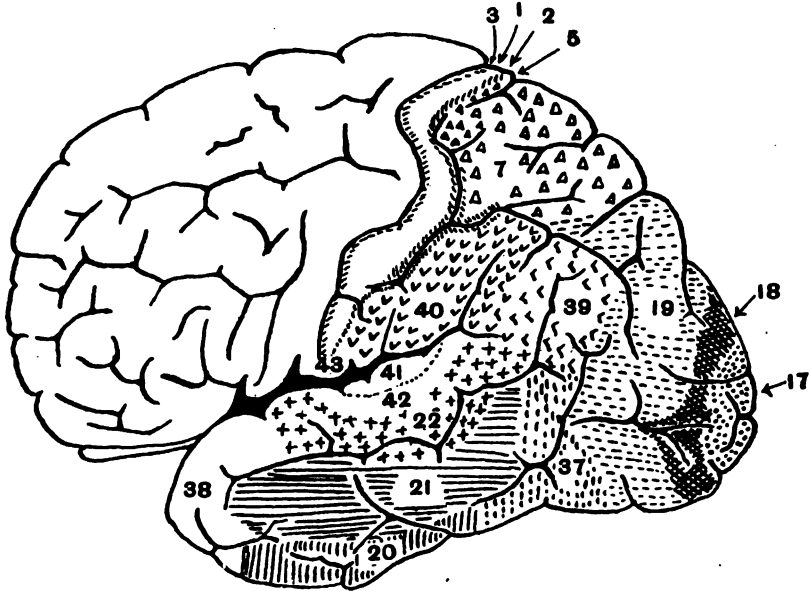


Fig. 16 Cortical areas in man, after Brodmann ('08).

merely a short connecting link between those portions of the interparietal which will become the inferior postcentral and the ramus occipitalis (*paroccipital* of Wilder). Referring to the account of interparietal in the Cercopithecidae, it will be remembered that this sulcus apparently gave off a branch, but in reality was itself continued upward, limiting the first annectant gyrus in front. This furrow forms the posterior limit of area 7 and a small, and doubtless varying extent of the ascending limb of the interparietal immediately in front, in the region where it is crossed by area 7, represents the sulcus interparietalis proprius. Its an-

terior limit is the second, inconstant, branch of the interparietal mentioned above lying between areas 5 and 7. In *Hylobates* this tiny bit of sulcus is usually markedly lengthened. The original small furrow which cut into the first annectant gyrus in front is more constant and well defined and appears here also as a continuation of the interparietal (*proprius*) in that just beyond it in the anterior part of the *ramus occipitalis* there is an annectant gyrus or the latter sulcus is distinctly shallower.

The terminal portion of the interparietal, the *ramus occipitalis*, lies within area 19, it may turn upward underneath the operculum, forming the posterior limit of the first annectant gyrus, as referred to above in *Macacus*. The same arrangement may occur in *Hylobates* or more commonly the main sulcus continues downward and backward and the furrow behind the first annectant gyrus joining the interparietal is new formed (Cunningham). In both cases there is marked off the typical paroccipital sulcus, while in the latter case, in which the *ramus occipitalis* seems to bifurcate, we have the sulcus *occipitalis transversus* of Ecker, which, since it is present with the lunate, cannot be derived from it. It is the continuation of the *ramus occipitalis*, i.e., the lateral branch of the transverse occipital, which forms the anterior limit of the peristriate area as we shall see later.

In the lower apes the variable and inconstant foldings described as superior postcentral probably cannot be looked upon as homologous with those which bear the same name in the *Simiidae* and in man. They are in relation with different areas being located farther forward in the lower types. A possible exception might be made in certain cases of *Semnopithecus* which is closest to the anthropoids, although it is readily conceivable than even in the lower apes a true sulcus *postcentralis superior* might appear. The fissure of this name in human anatomy is new formed and much younger and is in relation in front with area 2 and behind the areas 5 and 7. In *Hylobates* it is still quite variable, equivalent at times doubtless with the fissure of lower forms. It may be absent and even when well developed is usually separated from the inferior sulcus by an annectant gyrus or is in addition shallower. Within this area 7 above the interparietal, as a result of continued

growth here, a new sulcus, the superior parietal may arise, and its existence is related to the later specialisation and further subdivision of this area.

The increase of cortex below, within the concavity of the interparietal, has led to the appearance of many new sulci. The progressive expansion of area 7 has gradually forced the interparietal farther away from the mesial border and given it a more

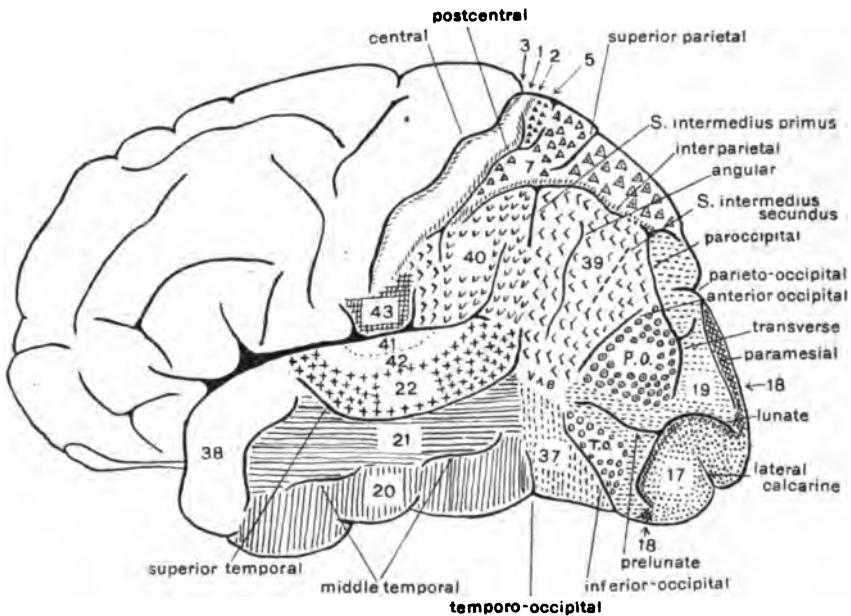


Fig. 17 Cortical areas in man, after Elliot Smith. V.A.B. indicates the visuo-auditory band; just above the sulcus interparietalis proprius is the visuo-sensory band. P.O. is the parieto-occipital and T.O., the temporo-occipital area.

sagittal course and the whole area instead of being a transversely placed strip of cortex has acquired a considerable antero-posterior extent. This is particularly so in the inferior lobule with the above mentioned results, but its effects are also felt below in the new course and relations already noted of the Sylvian and parallel sulci. In fact area 7 especially below the interparietal is, so to speak, a veritable storm-center, from which radiate in all direc-

tions energies and potentialities sufficient to entirely remodel the whole lateral aspect of the hemisphere behind the central fissure. Whether there are still resident in the brain of man, cortical areas possessed of such prophetic and prodigious possibilities is a question which we shall not attempt to answer.

At this point we shall have to leave the chart of the Gibbon as represented by Mauss and take up what we consider a more typical arrangement of fissures (figs. 8-10). Instead of pursuing a comparatively straight upward and backward course toward the parieto-occipital as it does in the lower apes, the superior temporal sulcus very frequently bends upward and even distinctly forward around the end of the Sylvian. There is thus left a large area between it and the interparietal and lunate and it is here that a very important fissure is found. In its new position the upward end of the parallel sulcus does not represent the anterior bounding sulcus of area 19 as it does in lower apes or may even in certain Gibbon brains, but that anterior limit is indicated by the above mentioned furrow which appears in the large area behind the end of the parallel sulcus. This furrow is the sulcus gyri angularis of Zuckerkandl ('04) and characterises his secondary gyrus angularis. It may be absent, particularly if the area in which it occurs is small, or if present it may be independent or form a (posterior) branch or even a continuation of the superior temporal from which it is often separated by a submerged gyrus. The interpretation of this sulcus is not quite clear. It might be considered as representing the extremity of the superior temporal, which, on account of the growth of the surrounding parts has become, in certain cases, detached. On the other hand one might look upon it as a new furrow, which owes its existence to the same factors which have wrought so many changes in this region, chief among which would be the new posterior end of the parallel sulcus. This view would be further supported by the presence of an annectant gyrus; the furrow would then be as pure an example of compensation as one could desire. We are inclined to believe that the difference in the two explanations is not so great as it seems. This sulcus, which might have received a name less apt to occasion confusion, is the anterior occipital sulcus of Wer-

nicke, or the ramus ascendens sulci temporalis medii of human anatomy.

The region below the interparietal is more difficult to deal with in the higher Anthropoids on account of the greater complexity both as regards areas and sulci. The factors involved are an increase and further differentiation of this part of the parietal area and to a less extent, similar changes in the preoccipital area with the effect of their increased growth upon the areas and fissures of the temporal region. On account of the changes in areas 7, the Sylvian and parallel sulci have been forced downward and rendered more horizontal. The complexity of the posterior ends of these fissures has been heightened, being usually expressed in bifurcations or an increase in the foldings in the neighborhood or by confluence with them. This applies especially to the parallel sulcus and with it the anterior occipital which to a certain extent is compensatory, or one might say vicarious, in character.

With an area of constant extent it is obvious that the effect of increased folding will be to lessen its dimensions, as seen on the surface, and further to vary to a certain degree, its location. The common, deep posterior or descending branch of the Sylvian is sufficient to accommodate within itself the posterior part of area 22, which is not only slowly decreasing in size but is in addition being crowded downward by area 7 and this branch will then appear as the boundary between these two areas. This process of infolding would have materially shortened the main stem of the Sylvian were an increase in length not necessitated by the increase in area 7 or brought about by its union with already existent furrows, but this new end of the Sylvian, in cases in which it is present, turns sharply upward as a rule and lies, not between 7 and 22, but within 7 and therefore we have termed it new. This ramus ascendens is an axial furrow, which may however be separate and occupy varying positions or be associated with other furrows which could be termed axial since they occur within a definite area. The anterior occipital occurs more often as an independent, well developed sulcus in the higher Simiidae. The newer sulci which come into relation with the parallel sulcus are either

within area 7, in a sense axial, or they contribute to the folding in of areas 21 and 22 as they gradually take up a position lower down on the hemisphere. It is not uncommon to find between the superior temporal and lunate a large curved fissure parallel with the latter, its upper end being formed by a long anterior occipital, the lower by a fissure which is often represented by the descending branch of the superior temporal, while the middle connecting piece, probably formed mechanically as a continuation of either of the others, is less constant. This furrow forms the anterior limit of area 19, with the exception of its lower segments which cut directly through this area; the significance of this will be noted later. A part of area 21 is submerged in front.

In this inferior parietal region other noteworthy features are constantly encountered which are to be considered as forecasting its future subdivision in man. Above the posterior end of the Sylvian and in front of the superior temporal there are often found furrows indicative not only of an increase in area but also of a suggested separation of area 7 into an anterior and posterior portion such as occurs in man. In some cases, even in *Hylobates*, there is a well defined offshoot from the interparietal cutting more or less deeply into the surface between the Sylvian and parallel sulci. Although by no means always present, it may be very well marked or apparently represented by a long branch from either of the two mentioned sulci or it may be independent. It is, to be sure, an individual variation but suggestive of a cortical development more nearly approximating the human condition the development of which is likewise an individual variation. A comparison of this region in the human and anthropoid brains makes it evident that we must seek in the latter the foreshadowing of the supramarginal and angular areas (or gyri) of the former. It is the typical development of this sulcus between these areas which constitutes the sulcus intermedius primus of Eberstaller.

The distribution of the preoccipital, or peristriate, area 19, on the first annectant gyrus has already been mentioned and it is essentially this condition which is preserved throughout the Primates. From this position, i.e., above the interparietal, Brod-

mann and Mauss find area 19 passing this fissure and extending as far forward as the superior temporal (or anterior occipital) as noted above. If these conditions held we should have the termination of the old interparietal, namely the paroccipital and the lateral branch of the transverse occipital, cutting through the area in question, which is, *a priori*, rather improbable or at any rate indicative of some potential difference on the two sides. (Compare that portion of the interparietal in the lower apes which becomes the sulcus interparietalis proprius.) That there is this potential difference we have grounds for believing. Mauss states that the structure of this area is not uniform throughout, Campbell considers the part in question (below the interparietal) as a part of his "common temporal area" (Chimpanzee), Flechsig finds a difference in the time of myelinization and Elliot Smith separates it off as the parieto-occipital area, whose anterior limit is also the same limit of that portion of area 19 from which it is derived, viz., the anterior occipital.

An examination of the charts will show that in all forms there is a zone of contact between area 22 and area 19 or its derivatives, which relation seems to be preserved. In Brodmann's charts this connection, in man, is between areas 22 and 37 the latter of which he considers as a differentiation of 19. Elliot Smith finds it as his "visuo-auditory" band and a similar condition is to be seen in the diagrams of Flechsig. Brodmann's area 37, occipito-temporalis, is the paratemporal of Elliot Smith, which latter writer also describes a further differentiation of area 19 as the temporo-occipital area.

With the development of the three areas mentioned above and their associated fissures, one behind the other, we have reached a condition long recognized in human anatomy as the supramarginal, angular and posterior parietal gyri. The fact that these areas are among the youngest of the whole primate cortex and a consideration of the mode of development of the related sulci and of the individual variations which characterize any and every relatively new structure, will suffice to explain the complexity and variability of this region which are found not only in man but in the higher anthropoids as well.

The sulcus occipitalis inferior must now be considered. In *Cercopithecus* and indeed in most of the Old World Apes, in which it also goes under the name of occipitalis lateralis or occipito-temporalis lateralis, it bears much the same relation to the striate area as does the lunate and like this is often markedly operculated. This being the case, it cannot be the inferior occipital as we shall use the term for the human brain and the same restrictions will be necessary in its application to the Simiidae. The anterior end of the sulcus in *Cercopithecus* lies within area 19. There develops, however, particularly in the higher Cercopithecidae, a short furrow parallel with, and at a varying distance in front of, the up-turned end of the inferior occipital, the Querfurche of Zuckerkandl ('04). In its further development it exhibits the tendency to extend backward toward the occipital pole below the inferior occipital or may reach the tentorial surface further forward. It may be found as an apparent dependency of the middle temporal or be broken up into anterior and posterior fragments. Throughout these forms it represents the anterior limit of the preoccipital, area 19. Like the sulcus lunatus there is buried in the upper operculated wall of the inferior occipital in these animals, the occipital or parastriate area, 18, and to this wall it is largely restricted since it forms only a narrow zone between areas 17 and 19. The fate which, in many cases in man, overtakes the lunate is shared to even a greater extent and appears phylogenetically much earlier in the case of the inferior occipital.

It is fascinating to follow the life history and vicissitudes of the lunate and its operculum in the Primate brain. From its first and inconstant appearance in relatively simple form in the Lemurs, it pushes itself rapidly forward, driven by the increasing striate cortex behind, which far surpasses any other area in extent and rate of growth; not until there are submerged under the advancing operculum the major parts of the occipital and preoccipital areas, whose cortex already begins to show various furrows, and until it has reached the enormous dimensions in *Cercopithecus* and *Cynocephalus* is there anything encountered to stay its progress. From this point it begins to decline and recede, owing not only to a reduction in the extent of striate cortex but

also to the commencing development of the parietal area in front. These two factors with their individual variations determine the form, position and relations of the lunate. In *Hylobates* and even in some of the higher *Cercopithecidae*, the tide has turned against it and while in Chimpanzee conditions may seem to be more or less in the balance, in Orang and Gorilla the process is still more advanced. The great sulcus, "Affenspalte" of *Cynocephalus*, for example, is still represented in man and it is determined and defined by the conditions exhibited in the various annectant gyri, not, according to Zuckerkandl as to its homology and morphological value, but solely as regards its form, position and relations.

The expansion of area 19 below the lateral end of the lunate has led to similar but more far reaching and radical changes in the inferior occipital sulcus of lower Primates. Originally, in its posterior part, an inferior bounding sulcus, sulcus infrastratus, it gradually loses this exact significance and with the recession of the striate area it does not, like the lunate, follow it, being possibly prevented mechanically by the interposed end of the lunate, and hence becomes broken up and tends to disappear. In *Hylobates* it is as a rule much reduced and inconstant and its recognition in the higher forms and in men, as a sulcus sublunatus, is difficult if not practically impossible. Some of the small grooves behind the lunate and below the lateral calcarine might be interpreted as its last remnants. Further observations will be required to determine its exact fate.

The sulcus referred to above as developing out of the Querfurche of Zuckerkandl which is commonly encountered in the higher *Simiidae* and in man, occupies an analogous but in no sense an homologous position in the occipital region. It would seem at first glance to be the inferior occipital of the human brain which forms the lateral limit of the peristriate area, but this sulcus in man does not represent this limit of the primitive area 19, if we suppose that the paratemporal area, 37, is derived from it since it is located between this area in front and the temporo-occipital behind. It may be that furrows representing the descending branch of the parallel sulcus, which cuts directly into area 19, can be brought into relation with the inferior occipital and that

the *Querfurche* becomes associated with the temporo-occipital (inferior temporal) group of sulci. It is clear that further investigation of this region is necessary before any definite statements can be made.

The same might be said of the small sulcus frequently found within the angular area, 39, the sulcus intermedius secundus of Eberstaller.

One more sulcus, or system of sulci, remains to be considered. The sulcus *praelunatus*, or better *occipitalis lateralis*, is an axial folding of a forward extension of the peristriate area. Immediately in advance of this and forming in a way a continuation of it is the "visuo-auditory" band of Elliot Smith, connecting the peristriate and superior temporal areas. It is not found by Brodmann but would occupy the uppermost part of the area 37, between it and area 39. It is the axial folding of this band, in Brodmann's chart this would occur between areas 37 and 39, particularly common in the human brain which constitutes the temporal-parietal sulcus.

The fissural pattern within the occipital region behind the lunate, and its significance has been fully elucidated elsewhere and we shall not consider the question further.

In attempting to understand the brain of man, and the same considerations hold to a lesser degree for that of the higher Anthropoids, one must constantly bear in mind the fact that one is dealing with individual cases, cases subject to individual variation to an extent not yet determined for any single species, *homo sapiens* not excepted. If variability is ever disconcerting it is here, for one has to reckon with a variability of the most subtle and elusive character, occurring as it does in the last and highest product of evolution where the range and complexity of variation is so great and our knowledge of its structural and functional manifestations, for even a single case, so slight. Bearing this in mind and remembering that we must interpret the foldings of the cortex in the light of our knowledge of the structure of that cortex, we may consider briefly certain conditions in man. It will be evident that with the knowledge at our disposal at present we can only hope to approximate the truth in our conception of the

surface markings of the brain, but with a constantly diminishing probability of error as our knowledge increases. Only after an exhaustive examination of the entire cortex could one begin to speak dogmatically and then only of the particular brain investigated. Such an herculean task is quite out of the question for any large series of brains and for the vast majority of those which come into our hands we must be content with an approximation of the true conditions.

We shall not take time here to discuss a number of separate cases but shall endeavor to apply the reasoning contained in the foregoing pages. Primitive types of configuration are naturally desirable and instructive, where one can sometimes find a strikingly pithecoïd pattern, and it is to one of these that we shall call attention.

Figures 18 and 19 represent the posterior portions of the right and left hemisphere of a Soudanese negress. The pattern is simple and clear, almost diagrammatic, all the typical fissures being present with very few others. Other primitive brains which have been recorded, Tasmanian, Australian, Egyptian, etc. would have served our purpose also. We shall base our discussion upon this brain, referring to the varying conditions found elsewhere.

The lunate sulcus is as typical as could be desired, especially on the right side. It is deeply operculated and in every way indicative of an unusually extensive area striata. Lunate sulci are not, as Cunningham supposed, unusual, a search through any work where a considerable series of brains is figured will reveal numbers of cases. Many can be found in the Swedish brains of Retzius ('96), more recently Murphy has published some very well marked examples, especially in the negro, and Elliot Smith has shown them in Tasmanian and Australian ('11) and in numerous Egyptian brains ('04). Although doubtless of little value as a racial characteristic, they are, when very well developed, suggestive of less advanced conditions in the surrounding areas which have allowed a typical lunate to be formed. One might confidently expect to find cases in any large series of brains from any source.

Depending on the position of the sulcus, whether near the mesial or lateral border or as in this case intermediate, the position of other furrows will vary, particularly the paramesial above, and which may be on the mesial surface, and the inferior occipital which may be in part on the tentorial surface. The position of the lunate is primarily determined by the relative development of the areas in front and by the extent of striate cortex. These

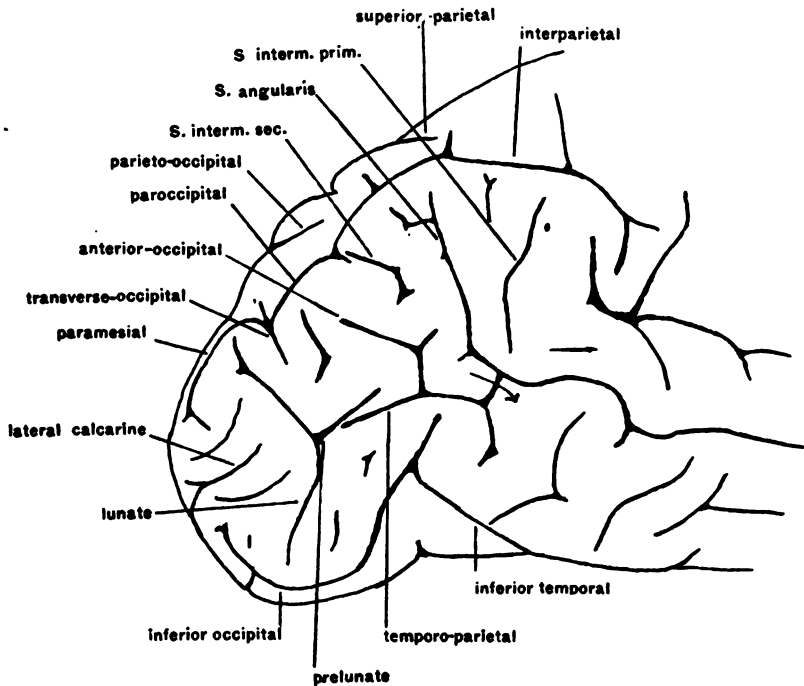


Fig. 18 Right hemisphere of a Soudanese negress.

factors may be such that the lunate is crowded far back, or may fail to develop or assume varying forms or be divided by a gyrus translunatus. With the shifting of the area striata there is associated a corresponding shifting of the furrows about it.

The prelunate or lateral occipital is best shown on the left side. From the changes in the areas above and below the forward projection of peristriate area of which this sulcus is an axial fold-

ing, its position will vary and may rise above or more usually below the center of the lunate. It will naturally be displaced backward with the recession of the striate area and may be present even though the lunate is wanting. Although variable it can usually be recognized and is important since it lies between two late differentiations of area 19, parieto-occipital and temporo-

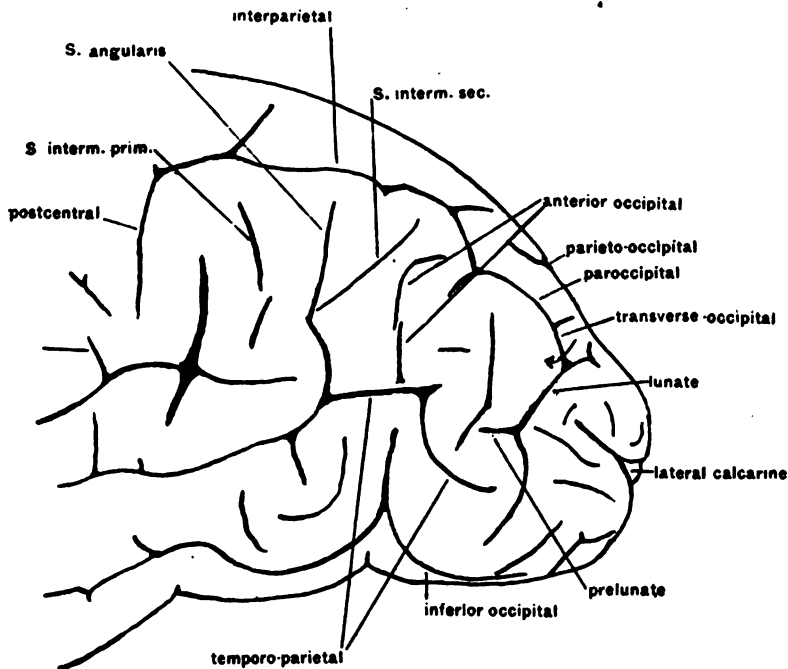


Fig. 19 Left hemisphere of Soudanese negress; same brain as figure 18.

occipital areas. It may become confluent with the temporo-parietal since they are both axial foldings in areas which lie one behind the other. When this occurs and the temporo-parietal unites with the superior temporal one has a not very infrequent condition in which the long tortuous furrow extends from the temporal almost to the occipital pole. In front of the anterior and inferior occipital sulci, this long furrow separates the parietal

from the temporal area (i.e., their derivatives), while behind it holds the same relations to the later differentiations of the pre-occipital area. We may note here that in all human brains, even the most primitive and pithecoïd, the distance between the lunate and superior temporal is always much greater than in any of the anthropoids. The reason for this has already been made clear. The superior temporal sulcus lies below a very productive area, the growth of which would tend to increase the distance between these two sulci.

The anterior occipital, limiting the parieto-occipital area in front, is more variable, since it is found in cortex that is, phylogenetically, very young. Its position will be largely influenced by the relations of the ramus occipitalis and this in turn by the peristriate area above it. It may be shifted downward in the same way and for the same reason as the lunate especially if the par-occipital is long. In extreme cases where the lunate has been pushed down to the lateral border the anterior occipital sulcus may be almost sagittally placed. It is usually found about opposite the parieto-occipital and may, as in this case be united with the temporo-parietal, or be independent. Frequently it is confluent with the middle temporal particularly when it is situated further forward.

The inferior occipital is quite typical on both sides, and essentially simian. It is the anterior and lateral boundary of the temporo-occipital and peristriate areas. It is a rather variable fissure: it may be found on the lateral or tentorial surface, its anterior end particularly tends to turn rather abruptly upward, which part may unite with the middle temporal, submerging in part between them the paratemporal area, 37. If lower down it can unite with the inferior temporal (temporo-occipital). Associated as it is with the peristriate area and through this with the striate its relations are closely connected with the condition of the lunate. This and the anterior occipital sulcus not infrequently unite to form a deep, vertical or curved fissure extending from the lateral border of the hemisphere upward toward the parieto-occipital. In shape it may resemble a lunate. This condition would

seem to occur particularly when the lunate is far back as if similar forces had caused a great transverse folding of the cortex.

The region above the interparietal will not detain us. Brodmann ('07) states that it can be subdivided into anterior and posterior halves, the line between the two being the superior parietal or precuneal sulcus, this would harmonize with the findings of Elliot Smith.

The inferior parietal region shows in this brain, more clearly on the right side, the three typical arcuate gyri, supramarginal, angular and posterior parietal. Such an arrangement is by no means the rule. This classical condition, with the addition of sulcus intermedius primus, between areas 39 and 40 and in front of the angular gyrus, and of the intermedius secundus within area 39 behind the angular, is however schematic rather than natural, and suggests a primitive condition. The furrows as they stand in the chart are not calculated to allow for any great increase in cortex and their number and relation is such that any slight increase is apt to lead to a distortion of the primitive markings. Area 40, whose posterior limit is the sulcus intermedius primus seems to be rather more constant than 39 behind it. This latter area presents two furrows, lying wholly within it, the angular or the secondary termination of the parallel sulcus, and the intermedius secundus, which is less constant. Whether or not these furrows may be related to differences in the different parts of the area, perhaps as yet potential, the future must decide. A multitude of fissural combinations may occur here and any attempted enumeration of them would, in the present state of our knowledge, be wasted time. It is possible however, here as in other regions to find the old landmarks, even although they may have forfeited entirely their original form, if strict attention be paid to the grouping and to the characters of the individual sulci, such as their depth and connections and the presence of annectant gyri. But in addition to this the influence of neighboring parts must be born in mind.

By the identification of homologous sulci, as far as such an identification is at present possible, we have mapped out homolo-

gous cortical areas and are in a position to compare the results thus obtained with each other or with any standard we may see fit to set up, representing average, racial or primitive conditions.

Individual variations in the cerebrum can only be variations of these cortical areas, doubtless in different degrees, which latter variations are in turn reflected in the varying fissural pattern. In comparing brains we should compare areas, functional subdivisions, rather than the largely mechanical results of their development. A mere comparison of gyri and sulci is vain and idle for any adequate understanding of the brain as an organ of mind. The best we can do at present is to institute comparisons between anatomical areas, in a few cases functional areas also, but not until we are able to speak throughout in terms of function shall we be able to appreciate the differences which we already recognize.

Without doubt there occur cases in which one can find anatomical relations, even macroscopic in nature, which can be brought into association with certain attainments or peculiar abilities of their possessors. But these, on account of our ignorance of the significance of many, even well defined areas, are as yet few in number. Much can certainly be done in a few selected cases by detailed histological examination of the entire cortex but for more rapid, general and practical purposes, we must have recourse to methods similar to the one outlined in this paper.

We would take occasion here to express our thanks to Prof. G. Elliot Smith in whose laboratory much of this work was done, for material and personal notes placed at our disposal.

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THE MEDULLA OBLONGATA OF LARVAL AMBLYSTOMA

C. JUDSON HERRICK

From the Anatomical Laboratory of the University of Chicago

FIFTY-SEVEN FIGURES

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INTRODUCTION

The morphological pattern of the medulla oblongata of vertebrates in general is well understood and the functional analysis of the cranial nerve roots, their bulbar centers and chief correlation tracts in different vertebrate types has been carried far enough to show that this pattern is fundamentally similar throughout the vertebrate phylum. The published observations on the internal structure of the urodele rhombencephalon are, however, meagre and quite inadequate to meet the requirements of a careful study of the bulbar connections of the higher regions of these brains.

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The great difficulty in getting adequate histological preparations of these brains is largely responsible for this situation; and, furthermore, the tissues themselves are organized in a very primitive fashion, so that, when the functional analysis is finally effected, the neurones of the gray substance can be separated into functionally defined groups with far less precision than in the brains of most fishes and amniote vertebrates and the localization pattern is found to be somewhat different in plan from that of the more highly differentiated brains.

The present study is based upon the brains of several species of lower urodeles, particularly *Necturus*, and a more detailed examination of that of *Amblystoma tigrinum* (Green). Both larval and adult stages have been studied, but this description will be confined to the larva of *Amblystoma* except when explicitly stated to the contrary. For most of this material I am indebted to the generosity of Professors Paul S. McKibben and Charles Brookover, who very kindly placed at my disposal a large number of microscopic sections of larval and adult brains prepared in diverse ways.

Kingsbury ('95) has given us a very exact and illuminating account of the medulla oblongata of adult *Necturus* and I have recently contributed ('14) a more detailed description of the cerebellum and its connections in this species. Many other authors have added important, though more fragmentary, data on the oblongata of urodeles. Kingsbury's description of the medulla oblongata and cranial nerves of *Necturus* is found to apply with very minor changes to larval *Amblystoma*. His account is based chiefly on Weigert preparations and, since in adult *Necturus* and in the larval stages of *Amblystoma* (where the architectural pattern is more simple than in the adult) many of the most important tracts are nearly or quite non-myelinated, further information is required regarding the connections of these non-myelinated fibers and the intrinsic neurones.

The fiber tracts of two specimens of larval *Amblystoma*, respectively 17 mm. (23 days after fertilization) and 38 mm. long, prepared by the silver reduction method of Ramón y Cajal and cut into transverse sections, were studied as completely as the

material permits. A wax model was constructed from the older of these specimens (figs. 1, 2, 3). The sections from which this model was constructed are 15μ in thickness and the scales attached to the figures give the serial numbers of the sections. Fifteen of these sections are illustrated in figures 4 to 18. In the descriptions of these sections their respective serial numbers are stated, so that reference to the scales attached to figures 1 to 3 will permit a ready localization of each section in its place in the model.

The fiber tracts of the 17 mm. specimen (23 days old) are not as fully developed as in the older one modeled, but so far as revealed they exhibit the same arrangement. Longitudinal sections of several *Amblystoma* larvae from 20 mm. to 60 mm. long prepared by Ramón y Cajal's method were also studied for additional control and various cytological methods were also used. Weigert preparations of a second specimen of 23 days shows that at this age few myelinated fibers are present in the oblongata, though some very coarse ones are found in the fasciculus longitudinalis medialis. I have studied an extensive series of Golgi preparations of larval and adult *Amblystoma*, comprising more than 150 specimens. The methods of Weigert and Ramón y Cajal have also been applied to the adult brain. The adult has not been studied as exhaustively as the larva, but so far as observed the adult in all fundamental relations resembles the larva.

In this study the sensory centers and their connecting tracts have been most carefully examined, though the motor apparatus has also received attention. The analysis even of the sensory apparatus is far from complete, but it is adequate to show the general arrangement and most important connections. This arrangement is simple and probably very primitive and it is hoped that this analysis may serve as a point of departure for studies in the evolution of the medulla oblongata of vertebrates.

Here, as in general throughout the urodele brain, there are few clearly differentiated gray centers, but the cell bodies of all the neurones retain their primitive positions in the central gray (stratum griseum), while all fiber tracts and synapses are found in the superficial stratum album. Occasional nuclei are found in

the white layer throughout the brain. In the medulla oblongata these appear darker in preparations stained by the method of Cajal than do the nuclei of the gray layer. In figures 4 to 18 the latter are drawn as light circles, while the nuclei of the white layer are drawn in solid black. The character of the cells to which these nuclei belong is unknown.

Within the stratum album, moreover, root fibers and other tracts are arranged in a definite pattern and the dendrites of the neurones of the stratum griseum extend outward among these tracts and the associated areas of neuropil, thus effecting their respective synaptic connections. The axones of these neurones also enter the stratum album and in this way the correlation tracts are formed. Not until the connections of the axones and dendrites of the individual neurones are fully known is it possible to understand their physiological significance; and since the mastery of these histological details is exceedingly difficult, our knowledge of the internal structure of the amphibian brain is still rather fragmentary. The application of appropriate neurological methods, however, reveals a very definite functional localization, within the stratum album, with a corresponding specificity in the related neurones of the stratum griseum. The analysis of these relations in the oblongata of *Amblystoma* is the purpose of this research.

THE SPINAL CORD

The spinal cord of urodeles has been described by several authors, most fully by Van Gehuchten ('97), most of whose observations on larval *Salamandra* I have confirmed in my own Golgi preparations of the upper segments of the spinal cord of larval *Amblystoma*. The literature of this subject has been fully reviewed by Van Gehuchten in the paper cited and need not be further considered here.

In the half grown larvae from which the present description is chiefly drawn the configuration of the gray and white substance of the spinal cord is quite different from that of the adult. The stratum griseum, containing the cell bodies of practically all of the neurones of the cord, is very wide and it extends quite to the dorsal

surface for a considerable distance on each side of the mid-dorsal plane. This is an embryonic character which is not preserved in the adult. The mid-ventral plane, on the other hand, is occupied by a thin layer of white substance containing the fibers of the strong ventral commissure.

The boundary between the gray and the white layers is an intermediate region poor in cells, but rich in dendritic processes and axones, many of which run tangentially to the gray layer. This is termed by Van Gehuchten the marginal zone of the gray substance. Within this layer are found occasional large spindle-shaped correlation neurones, from both ends of which massive dendrites arise and extend for long distances within the same layer, giving off branches which may spread through practically all parts of the white layer. Similar tangential neurones are found in the medulla oblongata (where the marginal zone of the gray substance is also present) and these probably represent the most highly specialized types of correlation cells.

At the periphery of the stratum album is a dense neuropil formed chiefly of fine ramifications of dendrites of correlation neurones. This is the plexus perimedullaris of Cajal, Lavdowsky and Sala and is termed the marginal zone of the white substance by Van Gehuchten. This layer is also present, though very unequally developed in different regions, throughout the brain, and within it the most important synapses appear to be developed.

The neurones which give rise to ventral root fibers occupy a ventral position in the gray layer and send large dendrites dorsalward within the marginal layer of the gray substance, from which branches are given off which reach practically all parts of the white substance of the same side. Three small neurones of this type with relatively short dendrites are seen in figures 17 and 18 (*col.v.*). The remainder of the gray substance contains correlation neurones of several diverse forms, some of which also send dendrites into all parts of the white substance. Many large neurones of the ventral parts of the gray substance send big dendrites through the ventral commissure to arborize in the stratum album of the opposite side. Whether any of these give rise to ventral root fibers I have not determined, and Van Gehuchten's

figures show no such dendrites crossing in the ventral commissure in *Salamandra*.

So far as appears from previous descriptions and from my own observations there is little evidence of any great capacity for functional localization in the spinal cord of these larvae beyond the general distinction between dorsal sensory and ventral motor regions. The anatomical arrangement suggests that in the normal functioning of the spinal cord, so far as concerns the larger neurones at least, any stimulus whatever discharging into the dendritic complex of the cord, whether derived from the dorsal roots or from any of the longitudinal fiber tracts of the white substance, would naturally call forth a total reaction of the entire body musculature such as the typical swimming reaction.

The earliest reflexes to appear in the spinal cords of these larvae have been shown by Coghill ('09) to be crossed reactions involving an ascending impulse in the dorsal tracts, a decussation in the ventral commissure of the upper levels of the cord or in the oblongata and a descending impulse in the ventro-lateral fiber tract of the opposite side. But in the half grown larvae here under consideration there is evidently present in addition to this primary connection a very complete mechanism for purely local reflexes within a single segment, including a direct synaptic connection of dorsal root fibers with dendrites of ventral horn cells of the same side, as well as an intricate system of intrinsic correlation neurones in each segment for local and remote reactions.

THE GENERAL STRUCTURE OF THE MEDULLA OBLONGATA

As the spinal cord passes over into the medulla oblongata the fundamental histological structure remains essentially similar, but there are important differences in the details of the functional connections of the constituent neurones. The sensory fibers of the roots of the cranial nerves which enter the oblongata are segregated into distinct functional systems, whose peripheral connections have been accurately determined by Coghill ('02). The root fibers of these functional systems as they enter the oblongata give to the white substance of the receptor region a definite pattern and the great ascending and descending correlation tracts simi-

larly establish a very precise regional functional localization in the remaining parts of the white substance. From this it follows that, although in the oblongata as in the cord the dendrites of many neurones of the gray substance spread very widely throughout the white layer, it is possible in all such cases to determine with a high degree of precision the functional character of the various fiber systems with which these dendrites effect synaptic relations. In the medulla oblongata, therefore, we have an exceedingly primitive generalized type of histological structure and at the same time a definite pattern of functional localization. The functional factors which determine this localization are represented structurally by an arrangement of peripheral nerve components and central fiber tracts which can readily be correlated with those of the mammalian nervous system. The functional composition of the cranial nerve roots is known to agree with that of other Ichthyopsida, and save for the lateral line roots with that of the Amniota as well. Though the central courses of these root components and of the associated correlation tracts are arranged according to a much simpler pattern than in any other vertebrate hitherto described, nevertheless the homologies of these tracts in higher brains can in most cases readily be recognized.

In the embryonic development of the neural tube of vertebrates the sulcus limitans marks the primary boundary between the dorsal afferent and the ventral efferent systems; and in general this boundary is more or less evident throughout life in the amphibian oblongata. In fishes and higher vertebrates the efferent centers are further divisible into a ventro-medial somatic column and a ventro-lateral visceral column, the latter including the pre-ganglionic sympathetic neurones for smooth muscles and glands (general visceral efferent system) and the motor nuclei from which are innervated the branchial and masticatory muscles (special visceral efferent system for the striated visceral muscles of the head). In these groups of animals the afferent centers are likewise divisible into a ventral visceral sensory column (the fasciculus solitarius and associated gray matter) and a dorsal somatic sensory column, the latter receiving the roots of the general cutaneous, VIII and lateral line nerves.

In half grown *Amblystoma* larvae the relations just described are seen except for some secondary shifting of parts, notably the ventral movement of a part of the somatic sensory column along the lateral surface of the oblongata so that the VIII and spinal V roots lie superficially and ventrally of the fasciculus solitarius (see figs. 13, 14).

The four fundamental columns of the oblongata are readily identified in both larval and adult *Amblystoma* so far as the central connections of the peripheral nerve roots are concerned. The somatic motor column contains the nucleus and roots of the VI cranial nerve, the fasciculus longitudinalis medialis and some other correlation tracts. The visceral motor column includes the motor nuclei and roots of the V, VII, IX and X nerves. Only the special visceral motor nerves for the striated visceral muscles of the jaws, hyoid and branchial arches are clearly differentiated; the general visceral efferent (preganglionic) components of these nerves for unstriated muscles and glands have not been identified, a feature probably to be correlated with the feeble development of the head part of the sympathetic system in urodeles (cf. Hoffmann '02). The visceral sensory system is represented in the portio intermedia of the VII nerve and in the visceral sensory components of the IX and X nerves. All of these fibers enter the fasciculus solitarius (fasciculus communis of Osborn) and terminate in synaptic relation with the neurones of the adjacent parts of the stratum griseum. The somatic sensory system is represented by the general cutaneous fibers of the V nerve and by the component of the X (and probably of the VII nerve also) which enters the spinal V root, also by the special sensory systems of the VIII and the lateral line roots of the VII and X nerves.

The components of the cranial nerves of *Amblystoma* and their peripheral distribution have been fully described by Coghill ('01, '02). Professor Coghill informs me that these descriptions were based upon large larvae 12 cm. long with functional external gills, controlled by the examination of the adult after metamorphosis. The roots of the cranial nerves of the younger larvae used in the present study are so similar to those of Coghill's specimens that a separate description will not be necessary. Norris ('13,

p. 285) in a recent description of the cranial nerve components of *Siren* notes a separate general cutaneous root and ganglion of the facial nerve. Coghill did not recognize such a root in *Amblystoma*, nor have I seen its peripheral course, though I have some evidence of its presence (see p. 361).

The motor roots of the V nerve emerge ventrally of the sensory roots; those of the VII nerve ventrally of all sensory VII and VIII roots and partly ventrally of the spinal V root and partly through it; the motor IX root fibers pierce the VIII roots; and the motor X rootlets emerge at various levels. The more rostral of these motor vagal roots emerge above the spinal V root, the middle ones through this root and the more caudal ones below it.

In the 38 mm. larva from which figures 1 to 18 are drawn there are seven distinct roots of the vagus. The first includes the dorsal and ventral lateral line roots, which enter the oblongata close together at the same transverse plane as the IX roots (fig. 3). The second and third roots contain visceral sensory and visceral motor components. These two roots constitute the second root of Coghill ('02, p. 283). The fourth and fifth roots contain visceral sensory fibers (entering the fasciculus solitarius), somatic sensory fibers (entering the spinal V root) and visceral motor fibers; these are Coghill's third root. The sixth and seventh roots are exclusively visceral motor and might be further subdivided. They form Coghill's fourth root, which he says may arise by as many as five distinct rootlets.

Figure 3 shows diagrammatically the central courses of the sensory components of the V to X cranial nerves as projected upon the lateral surface of a model of the medulla oblongata. This arrangement is preserved without essential modification in the adult, though with some changes in the form relations and much additional complexity of detail.

Examination of the model from above (fig. 1) shows a configuration not unlike that which I have described ('14) in adult *Necturus*. The wide rhomboidal fossa is contracted in the upper vagal region; passing from this region forward, it gradually expands in the regions of the IX, VIII and VII nerves; and between the V roots and the cerebellum abruptly dilates to form the

lateral recesses, whose walls form the auricular lobes of the medulla oblongata.

In the flat and wide medulla oblongata of *Necturus* I have described ('14, figs. 1, 2, 3, 15, 16) in the floor of the fourth ventricle a sulcus limitans separating the motor and sensory columns, and farther laterally a sulcus lateralis which forms the medial boundary of the area acustico-lateralis. In larval *Amblystoma* these sulci are in some places obscurely evident, but on account of the narrower and higher form of the oblongata in these larvae they are not so simply related to the underlying regions, and in the adult the deviation from the form seen in *Necturus* is still greater.

In the caudal part of the fourth ventricle there is a shallow sulcus limitans (fig. 2, *s.l.*), which separates the ventral motor lamina from the dorsal sensory lamina; and in the sensory lamina there is an obscure visceral lobe (fig. 2, *lob.vis.*) formed by the fasciculus solitarius and its associated gray matter (figs. 14 to 17), which clearly corresponds with the so-called lobus vagi of fishes. Near the lower end of the fourth ventricle this visceral lobe arises up behind the caudal end of the area acustico-lateralis almost to the dorsal border of the rhomboidal lip (figs. 2, 17).

In the rostral half of the oblongata the massive wall on each side consists of a horizontal lamina which coincides approximately with the motor region, and a vertical lamina which is composed chiefly of the area acustico-lateralis, but the sulcus separating these two laminae does not coincide exactly with the embryonic sulcus limitans. The motor VII nucleus, like the nucleus ambiguus, lies far medial, but the motor V nucleus (fig. 1, *nuc.V.m.*) has been crowded lateralward by the great subcerebellar tegmental eminence (fig. 2, *em.s.t.*) and is in part overlapped by the great sensory trigeminal nucleus which forms the greater part of the eminentia trigemini (fig. 9).

The area acustico-lateralis forms the rhomboidal lip for nearly the entire length of the oblongata, from the tip of the auricular lobe almost to the calamus scriptorius. It is defined as the area which receives root fibers of the VIII and lateral line nerves and its extent may be seen at a glance in figure 3. As in *Necturus*, it

shows a distinct ventricular swelling at the levels of the superficial origins of the VII and VIII nerves, and also an anterior lobe which forms the lateral wall of the recessus lateralis.

The body of the cerebellum is larger in this larva than in adult *Necturus* and it occupies the entire rostral end of the auricular lobe and here root fibers of the cranial nerves extend forward from the area acustico-lateralis into the body of the cerebellum, a condition which has not been demonstrated in *Necturus*. The roof of the wide recessus lateralis is entirely membranous and plexiform. No massive cerebellar tissue is developed in the mid-dorsal plane except the fibers of the decussatio veli, which has the same components as I have already described ('14) for *Necturus*. Further details regarding the development and structure of the cerebellum of *Amblystoma* are reserved for a later communication.

The isthmus rhombencephali is not well developed ventrally, the massive fiber tracts of the pedunculus cerebri, lemniscus, etc., forming a strong ventro-lateral ridge between the rhombencephalon and mesencephalon. But dorsally the isthmus is marked by a deep total fold of the brain wall within which lies the IV nerve (figs. 1 and 2). The caudal lip of this fold is formed by the body of the cerebellum; the rostral lip by the tectum mesencephali.

The commissura tecti is feebly developed except for the massive posterior commissure at its rostral end. The caudo-lateral aspect of the tectum shows a well defined eminence, the nucleus posterior tecti (fig. 1, *nuc.p.t.*), and on the ventricular surface there is a corresponding evagination of the optocoele, the recessus posterior mesencephali (fig. 2, *r.p.m.*). The tectum mesencephali is bounded ventrally on the ventricular surface by a very shallow depression, which probably represents the position of the sulcus limitans, and below this is the motor tegmentum. The latter consists of two very distinct parts separated by a narrow and very deep sulcus which extends forward and downward from the ventral end of the recessus posterior mesencephali. Below this is the massive eminentia subcerebellaris tegmenti (fig. 2, *em.s.t.*). The ventral commissure system is well developed throughout the entire extent of the midbrain and oblongata, and is evidently very complex.

THE SENSORY ROOTS OF THE CRANIAL NERVES

The sensory root fibers which enter the medulla oblongata of *Amblystoma* in general bifurcate into ascending and descending branches immediately upon entering the stratum album, these ascending and descending root fibers being arranged in separate fascicles related with their respective roots. These fascicles have a definite arrangement which is maintained without essential modification throughout most of the length of the oblongata, the arrangement being such that the less highly specialized and presumably more primitive systems lie farther ventrally than the more highly differentiated systems (figs. 3 and 7 to 16).

The only important deviation from this rule is presented by the visceral sensory fascicle of root fibers, viz., the fasciculus solitarius (fasciculus communis of Osborn). Comparative anatomy and comparative embryology show that the fasciculus solitarius and its nucleus primitively lie ventrally of the spinal V root and its nucleus, an arrangement which has been secondarily modified in urodeles by a ventral movement of spinal V and VIII root fibers so that they come to lie superficially and ventrally of the visceral sensory root fibers and primary centers. The secondary visceral tract here, as in fishes, retains its primitive position below the spinal V root (figs. 7 to 16, *tr.v.a.*).

Within the sensory lamina of the oblongata the ascending and descending fibers of the chief sensory V root form the most ventral fascicle of the series. Immediately dorsally of these lie the two fascicles of VIII root fibers. The visceral sensory fibers of the VII and IX nerves pierce the fascicles of VIII fibers to enter the brain. The most rostral visceral sensory rootlets of the X nerve also pass through the VIII root fascicles, but the more caudal vagal rootlets of this system enter farther ventrally through the spinal V root. All visceral sensory fibers enter the fasciculus solitarius and here some (perhaps all) of them divide into ascending and descending branches. Unlike the other fascicles of root fibers, the fasciculus solitarius for its entire length lies deeply embedded within the substance of the oblongata, between the stratum griseum and the VIII roots. Dorsally of the fasciculus solitarius and VIII roots is a longitudinal tract of correlation

fibers, tract *b* of Kingsbury. Above this are found in sequence the ventral lateralis X root, the ventral lateralis VII root, the dorsal lateralis X root, the middle lateralis VII root, the longitudinal correlation tract *a* of Kingsbury, and the dorsal lateralis VII root.

The detailed relations of the somatic sensory roots of the cranial nerves will next be described. This will be followed by a description of the visceral sensory roots of these nerves and of the correlation neurones and fiber tracts with which all of these various roots stand in physiological relation.

The lateral line roots of the facialis. These form the dorsal VII (VIIb) of Strong ('95) and Kingsbury ('95) and VII u and 1 of Osborn ('88). Coghill ('02, p. 217) describes the lateralis VII root of old larvae and adults of *Amblystoma tigrinum* as entering the brain in four subdivisions, of which the first and third, counting from the dorsal side, lie farther caudad than the other two. The fibers of the first and third of these subdivisions come from the ramus mentalis VII via the truncus hyomandibularis; those of the second subdivision come from the ramus ophthalmicus superficialis VII; and those of the fourth come from the ramus buccalis VII.

In the 38 mm. larva the entire lateralis VII root complex enters the brain in one transverse plane (fig. 11) and I am not able to identify all of Coghill's four subdivisions with certainty. I recognize three roots, defined with reference to their relations with other fiber tracts of the oblongata. These are, counting from the dorsal side, (1) the dorsal root (*r.VII.l.l.d.*), entering dorsally of Kingsbury's tract *a*; (2) the middle root (*r.VII.l.l.m.*) entering between tract *a* and the dorsal lateralis X root; (3) the ventral root (*r.VII.l.l.v.*), entering ventrally of the dorsal lateralis X root and dorsally of the ventral lateralis X root and tract *b* of Kingsbury. The entire complex lies above and enters somewhat rostrally of the VIII roots.

The dorsal lateralis VII root is composed of finer fibers than either of the other two. These bifurcate immediately upon entrance into the oblongata and both the ascending and the descending branches terminate in an area of neuropil which forms the

most dorsal part of the white substance. This area was seen in *Necturus* by Kingsbury ('95, p. 187), who termed it the dorsal island of alba, and by Norris in *Amphiuma* ('08, p. 536) and in *Siren* ('13, p. 283), by whom it was compared with the lobus lineae lateralis of fishes. This 'dorsal island,' in both the 17 mm. and the 38 mm. larva, receives fibers only from the dorsal lateralis VII root, so far as I have observed, and its position is not marked by an external eminence. In the adult, however, it is much enlarged, and with the associated part of the stratum griseum, it completely fills a large eminence bordering the tenia of the fourth ventricle. In the adult, moreover, it receives large fiber bundles from the middle and ventral lateralis VII roots within the white substance of the oblongata. The entire structure in the adult much more strongly resembles the lobus lineae lateralis of the generalized fishes than in the young larva or the adults of the lower urodeles described by Kingsbury and Norris.

In the 38 mm. larva the ascending and descending branches of the dorsal lateralis VII root fibers extend only a short distance before terminating in the associated neuropil, their rostral limit being at the level of the V roots and their caudal limit at the level of the IX roots. The fibers of all of the other sensory roots which enter the oblongata extend much farther both rostrad and caudad.

The dorsal lateralis VII root of this description is evidently the same as the root VII b¹ of Strong ('95) and Kingsbury ('95). The root VII b² of these authors includes my middle and ventral lateralis VII roots.

Since the fibers of the dorsal lateralis VII root are smaller than any others of this system in these young larvae (this is not true in the adult), their peripheral course can be followed with ease. When this root is followed peripheralward from the oblongata, some of its fibers are seen to turn directly ventralward to enter the hyomandibular trunk for the ramus mentalis VII (the urodele equivalent of the teleostean r. mandibularis externus VII); but clearly in these larvae most of the fibers of this root enter the anterior division of the lateralis VII root complex to distribute in both the ramus ophthalmicus superficialis VII and the ramus

buccalis. It follows that in the larva, as in the adult, the "dorsal island," or lobus lineae lateralis, receives fibers from each of the three peripheral rami of the lateral line VII system.

The middle lateralis VII root enters the oblongata ventrally of Kingsbury's tract *a*. The ascending branches of its fibers pass forward parallel with and deeper than those of the dorsal lateralis X root and some of them reach the extreme rostral end of the auricular lobe. The descending branches also occupy a deep position adjacent to the central gray for most of their course, being readily followed in horizontal sections back to the caudal end of the area acustico-lateralis in the mid-vagal region. Peripherally the fibers of this root can clearly be seen to enter both the anterior and the posterior divisions of the lateralis VII root complex.

The ventral lateralis VII root in these larvae is larger than the other two roots and its ascending and descending branches form big tracts, each composed of several fascicles, lying between the dorsal and ventral lateralis X roots. The ascending fibers reach the rostral end of the auricular lobe and the descending fibers pass backward to the caudal end of the area acustico-lateralis. This root is also distributed peripherally to both the anterior and the posterior divisions of the lateralis VII root complex. In the case of the anterior divisions of the middle and ventral lateralis VII roots, it is not clear whether their fibers distribute to both the ramus ophthalmicus superficialis VII and the ramus buccalis. Apparently this is the case. The evidence, therefore, strongly suggests that each of the three lateralis VII roots receives fibers from each of the three great lateral line rami of the facialis.

The anterior lateralis VII ganglion (giving rise to the ramus buccalis and the ramus ophthalmicus superficialis VII) in these larvae is closely applied to the dorsal surface of the trigeminal ganglion, and along the plane of contact there is some mingling of the cells of these two ganglia. The cells of the lateralis ganglion are of large and medium size. Most of them are typical unipolar T-form neurones (fig. 46, *u.p.*) of very simple form, the single process very soon dividing into centrally and peripherally directed branches. Amongst these unipolar neurones, however, are found much less numerous bipolar neurones (*b.p.*), the central

and peripheral processes arising from opposite poles of the cell body, and an occasional transitional form in which the two processes arise close together from the same side of the cell body. The single process of the unipolar cell is always very slender, but beyond the T-form division both central and peripheral branches may increase in size very abruptly, the axones of some of these lateralis fibers being very large. The fine fibers of the dorsal lateralis VII root which enter this ganglion in general connect with the smaller cell bodies. The bipolar cells may be either large or small, the larger bipolar cells being related to some of the coarsest axones, though in this case also the fibers are greatly reduced in size before connecting with the cells.

The posterior lateralis VII ganglion (of the ramus mentalis VII) has the same internal structure as the anterior ganglion, save that no bipolar neurones were observed, either among the large cells related to the coarse fibers or among the much less numerous cells of medium size related to the fine fibers.

The lateral line roots of the vagus. There are two of these roots, dorsal and ventral, which unite immediately external to the brain to form the first vagal root in the enumeration on page 351. These roots by some neurologists have been associated with the IX nerve (IX¹ of Osborn, the first vago-glossopharyngeal root of Strong, IX¹⁺² of Kingsbury, X 1 of Coghill). The fibers of the lateralis component of the vagus are for the most part very coarse, with a few of fine calibre scattered among them. Their ganglion is composed of very large and medium sized neurones, most of which are unipolar, like those of the lateralis VII ganglia, only an occasional bipolar neurone being observed.

The lateralis X fibers separate, before entering the oblongata, into dorsal and ventral roots which enter respectively dorsally and ventrally of the ventral lateralis VII root. Each of these root fibers divides into ascending and descending branches immediately upon entering the oblongata, the ascending and descending dorsal and ventral fascicles maintaining very nearly the same relations to the other tracts of the oblongata as at the superficial origins of their roots. Some of the descending fibers of both fascicles reach to the extreme caudal end of the area acustico-

lateralis, and some of their ascending fibers reach to the extreme rostral end of the auricular lobe, where they end in intimate association with those of the medial and ventral lateralis VII roots. Between the V and VII roots many fibers of the ventral lateralis X root are seen in horizontal sections to turn and enter fascicles of arcuate fibers. They cross to the opposite side of the oblongata and their farther course is unknown.

The roots of the VIII nerve. The fibers of this nerve enter the oblongata by two imperfectly separated roots (dorsal and ventral) each containing fine fibers with some very coarse ones mingled with them. Peripherally also the coarse and fine fibers seem to be mingled in the various rami, but no attempt has been made to determine their distribution. The VIII ganglion is made up of very small cells, with some of medium size, all of which are bipolar. The two VIII roots enter the brain dorsally of the spinal V root and chiefly ventrally of the visceral sensory VII root, though some fibers of the dorsal root enter above the visceral sensory root (fig. 12).

The fibers of both roots divide immediately within the oblongata (fig. 48) and two fascicles of VIII root fibers may be distinguished for a long distance cephalad and caudad of the superficial origin of the nerve, derived respectively from the dorsal and ventral VIII roots. The fibers of the ventral fascicle run a longer course within the brain than do those of the dorsal fascicle. Mauthner's cell lies at the entrance of the VIII roots and two of its chief dendrites pass outward among the entering root fibers quite to the surface of the oblongata, one above and the other below the visceral sensory VII root (figs. 12, 53).

The dorsal and ventral ascending tracts of VIII root fibers remain distinct as far forward as the superficial origin of the V roots, beyond which they can no longer be separated. The combined root, of rather fine fibers, can readily be followed to the extreme rostral end of the auricular lobe, where, much reduced in size, it turns abruptly dorsalward (fig. 6) to terminate in the ventral part of the body of the cerebellum. A residue of the lateralis VII roots can be followed almost as far dorsally as these VIII fibers, ending in the same vicinity farther laterally.

The descending dorsal and ventral VIII roots remain distinct as far as the level of the second vagus root, beyond which they can no longer be separated, the mixed root continuing to or beyond the caudal end of the area acustico-lateralis. Below the level of the fourth vagus root these fibers become mingled with those of the fasciculus dorso-lateralis of the spinal cord and it has not been possible to determine their caudal termination. Wallenberg ('07) in the frog traced descending VIII root fibers downward as far as the sixth spinal segment.

Some of the VIII root fibers at their entrance into the oblongata are very coarse and some are fine. At their division within the oblongata the coarse fibers divide into branches of unequal size, the finer branches being directed rostrad (fig. 51) and the coarser branches caudad. From this it follows that the ascending VIII root fibers are all of fine calibre, while the descending fibers are partly coarse and partly fine.

A considerable number of VIII root fibers pass directly medially in the marginal zone of the gray substance and apparently decussate in the ventral commissure, but I have not been able to determine their ultimate distribution.

The general cutaneous roots of the V, VII, and IX nerves. The trigeminal fibers of this system arise from neurones of the semilunar or Gasserian ganglion. This ganglion is composed of neurones of various sizes, large, medium and small, all of which so far as observed are unipolar (fig. 45). The larger cell bodies are connected with the larger root fibers.

Some details of the mode of termination of the collaterals of the V root fibers are seen in figure 49. Golgi preparations show further (fig. 50) that the larger fibers of the sensory V root take a deeper position within the oblongata before dividing into ascending and descending branches. The ascending branches (which are of smaller calibre than the descending and in the adult are unmedullated) pass forward to the rostral end of the auricular lobe (figs. 3, 6). The finer fibers of the sensory V root also bifurcate into ascending and descending branches and take a more superficial position in the oblongata. The descending branches of these fibers throughout the length of the spinal V root are

intimately associated with the ascending secondary visceral tract (figs. 5 to 16, *tr.v.a.*) and give off innumerable delicate collaterals among the fibers of the latter tract. The indications are that these fine trigeminal fibers effect synaptic connections with the same neurones as do those of the ascending secondary visceral tract, probably for tactual-gustatory correlations (see figs. 25, 27, 31).

In figures 9 and 54 are seen scattered coarse arcuate fibers passing from the vicinity of the V root through the stratum album into the ventral commissure. Many of our preparations suggest that these are crossed sensory root fibers terminating in the motor V nucleus of the opposite side, but the relations are confused here by the presence of other similar fibers which clearly connect with the area acustico-lateralis farther dorsally.

In a number of longitudinal sections by the methods of Cajal and Golgi I have seen evidence of a few fibers from the VII root complex entering the spinal V root. In one Golgi impregnation such a fiber is clearly defined for a long distance. This fiber bifurcates into ascending and descending branches, both of which enter the spinal V root. These fibers probably represent the general cutaneous component of the facialis described by Norris ('13, p. 285) in Siren, though I have not succeeded in following them peripherally or recognizing their ganglion, and my observations are so few as to require further control.

Fibers of the general cutaneous system enter the oblongata by two of the vagus roots (see p. 351) and immediately divide into ascending and descending branches accompanying the spinal V fibers (figs. 16 and 48). In the frog Wallenberg ('07) finds that spinal V root fibers descend as far as the eighth spinal segment.

The mesencephalic V root. I have previously recorded some observations upon the central course of this root in *Necturus* and *Amphiuma* ('14, pp. 10 and 14). In these species I found that the fibers of the mesencephalic V root pass forward to the tectum in company with the fibers of the tractus spino-tectalis to connect with the cells of the nucleus magnocellularis tecti, chiefly of the same side but partly of the opposite side, the latter fibers crossing in the decussatio veli in company with those of the cerebellar

commissure and IV nerve. Mention was also made of a coarse fibered tract seen by Osborn ('88) and Kingsbury ('95) which appears to accompany the mesencephalic V fibers and can be followed caudad as far as the roots of the VII and VIII nerves. Norris ('13, p. 269) describes these fibers in *Siren* as a portion of the more posterior of the two rootlets by which the mesencephalic V fibers enter the brain, this posterior rootlet dividing into anteriorly and posteriorly directed tracts. The latter tract passes backward at the ventral border of the gray matter "and can be traced as far posteriorly as the level of the root of the seventh nerve."

This description of Norris I confirm, and add the further observation that in *Amblystoma* not only do the bundles of root fibers of both the anterior and posterior rootlets divide into ascending and descending tracts, but the individual fibers of the mesencephalic V root bifurcate to enter these tracts. This can be very clearly seen in several of my specimens in horizontal Cajal preparations. Figure 54 illustrates such a horizontal section taken at the level of the bifurcation of several of the mesencephalic V root fibers. These root fibers are coarser than any others in the preparation and can readily be separately followed for their entire length throughout the series of sections. The section figured is tangential to the ventral border of the stratum griseum and the adjacent section dorsalward includes neurones of the motor V and motor VII nuclei at the locations designated,

Some of our preparations show slender collateral branches of the mesencephalic V root fibers entering the motor V nucleus, but these are not visible in the preparation figured. The descending branches are, however, clearly shown, these branches being in many cases as large as the stem fibers from which they arise. They pass backward and inward and arborize among the dendrites of the motor VII neurones. The ascending branches of these root fibers can readily be followed to the tectum mesencephali, as in *Necturus*, few of these fibers crossing in the *decussatio veli*. In the tectum these very coarse fibers form the deepest elements of the stratum album, from which they turn abruptly inward one at a time to enter their respective cell bodies of the nucleus magno-

cellularis tecti (figs. 55, 56). These neurones are scattered throughout the length of the tectum mesencephali and at various levels in the substance of the stratum griseum. Very many of these neurones are apparently perfectly impregnated in our Cajal preparations, but in no case have I ever seen any indication of dendrites or collateral branches arising either from the cell body or from any portion of the mesencephalic V root fiber within the mesencephalon.

A diagram of the central relations of the mesencephalic V root of larval Amblystoma, as seen from the dorsal side, is shown in figure 57.

The morphological and physiological significance of the mesencephalic V root have been much discussed. A large number of observations, particularly those of Johnston ('05, '09) and Willems ('11) make it appear probable that this root is concerned with muscular sensations from the trigeminal muscles and that the neurones of the mesencephalic V nucleus are comparable with spinal ganglion neurones. In these Amblystoma larvae the superficial resemblance between these neurones and the larger ones of the semilunar ganglion of the V nerve is indeed very striking (cf. figs. 55 and 56 with fig. 45), though without further comparative cytological and embryological study it would be obviously unwise to stress this point. Upon this interpretation of the mesencephalic V nucleus, the single process arising from these unipolar neurones could be compared with that of the neurones of the semilunar and spinal ganglia. Just as the single process of the spinal ganglion cell divides in the form of a T into peripherally and centrally directed branches, so the single processes of the mesencephalic V neurones divide just within the superficial origin of the root into peripherally and centrally directed processes, the latter of which terminates in synaptic relation with the motor V and motor VII nuclei.

A quite analogous relation is presented by the transitory giant ganglion cells of Rohon and Beard in the spinal cords of various larval Ichthyopsida. In some cases, as in *Lophius*, these giant cells persist up to adult life. In very young larvae of Amblystoma these giant cells have recently been shown by Coghill ('14)

to be bipolar, with the peripheral process directed outward into relation with both the skin and the myotomes. The myotomes, however, have an independent motor innervation and these muscular branches from the giant cells are unquestionably concerned with the muscle sense in relation with the swimming reflex. The physiological as well as the anatomical significance of these relations have been very completely investigated by Coghill. It is known, furthermore, that these giant cells of the spinal cord are derived from the neural crest, some of whose cells are enclosed within the spinal cord, while others migrate outward to form the spinal ganglia.

I have some observations which suggest that in *Amblystoma* the cells of the mesencephalic V nucleus are likewise derived from the neural crest, but my series of stages of these very young embryos is not sufficiently complete to enable me to verify this supposition and the question requires further study.

Upon the hypothesis that the mesencephalic V root serves the proprioceptive function of sensori-motor reflexes from the muscles of the head, the functional advantage of the arrangement described is obvious; for we have here provision for the most direct possible connection between the sensory fibers derived from the muscles of the head and the motor nuclei from which these muscles are innervated.

The visceral sensory roots of the VII, IX and X nerves. These roots are made up exclusively of very fine fibers. The neurones of the ganglion geniculi of the facialis are of small or medium size and so far as observed are unipolar, though but few of these neurones are clearly impregnated in our preparations. All of the root fibers of this system at once enter the fasciculus solitarius (fasciculus communis of Osborn), which is composed almost exclusively of these root fibers.

The fasciculus solitarius is anatomically the most distinct of all the tracts of the oblongata. Its fibers are fine and many of them (perhaps all) bifurcate in the usual way into ascending and descending branches immediately upon entering the oblongata from the periphery. It lies dorsally and medially of the VIII roots and ventrally and medially of Kingsbury's tract b. The

slender prefacial portion of the fasciculus is composed chiefly of the ascending branches of root fibers from the VII, IX and probably also the X nerves. In horizontal longitudinal sections of both larval and adult brains it can easily be followed forward nearly to the extreme rostral end of the auricular lobe. At the level of the superficial origin of the V nerve (fig. 9) it lies dorsally of the V root fibers and internally of the VIII fibers, a relation which is preserved to the rostral end of the auricular lobe.

In larval *Amblystoma* the fasciculus solitarius has no apparent connection with the V nerve, and if any V root fibers enter it their number is certainly small. In the adult a few medullated fibers are seen in horizontal sections to leave the sensory V root at its superficial origin and to pass inward to the fasciculus solitarius, where they turn caudad within or in close proximity to this fasciculus. In adult *Cryptobranchus alleghaniensis* these fibers from the V root are more numerous than in *Amblystoma*.

The rostral terminus of the prefacial fasciculus solitarius is an ill defined deep-seated area of neuropil internally of the terminus of the V and VIII root fibers in the auricular lobe. In figure 6 it lies in an undesignated area immediately dorsally of *r.V.* In the adult this neuropil lies close to the stratum griseum rostrally of the motor V nucleus and laterally of similar large neurones in the caudal part of the *emineptia subcerebellaris tegmenti* which is the direct forward continuation of the motor V nucleus. The latter neurones correspond in position to the locus coeruleus of mammals and may be homologous with this nucleus. All relations of the prefacial fasciculus solitarius are much clearer in the adult brain than in the larva, but as I have no satisfactory Golgi impregnations of this region I am not able to give further details. This terminal neuropil of the fasciculus solitarius lies a short distance caudad and laterad of a larger and more clearly defined area of neuropil in the isthmus which receives the ascending secondary visceral tract (see p. 373 and figs. 5, 6, 52, *tr.v.a.*) and is apparently the amphibian equivalent of the teleostean "Rindenknotten" (Mayser '81), or superior secondary gustatory nucleus (Herrick '05), but there seems to be no direct connection between the fasciculus solitarius and this secondary visceral nucleus.

Following the fasciculus solitarius caudad, it is seen to be enlarged by successive additions from the VII, IX and X nerves (figs. 3 and 12 to 18) and in the vagus region there is an ill defined ventricular eminence formed by the thickening of the stratum griseum adjacent to the fasciculus solitarius (figs. 2, 14 to 17, *lob. vis.*). The rostral end of this visceral lobe is separated from the overlying area acustico-lateralis by a shallow sulcus, but caudad of the latter area the visceral lobe rises up quite to the dorsal border of the massive wall of the fourth ventricle. In this lower region of the oblongata the fibers of the fasciculus solitarius also rise up within the substance of the visceral lobe and, just below the calamus scriptorius, the remaining fibers decussate in the commissura infima Halleri, near which they terminate in the commissural nucleus of Cajal in the same way as in fishes and mammals (figs. 17, 18). There is also a decussation of fibers from the somatic sensory column in the commissura infima (fig. 18, *com.i.s.*), so that the relations in this region closely resemble those which I have previously described ('08) in teleosts. Wallenberg ('07) in the frog describes fibers of the spinal V root entering the commissura infima and fibers of the fasciculus solitarius descending to the third spinal segment.

Summary. The preceding account shows that most of the sensory root fibers of the cranial nerves which enter the medulla oblongata of larval *Amblystoma* immediately divide and extend in ascending and descending directions throughout the greater part of the length of the oblongata. These fibers give off frequent collateral branches, thus effecting synaptic relations with dendrites of neurones of the adjacent gray substance throughout their entire extent. In this respect they resemble the root fibers of the spinal nerves, though here there is a well defined grouping of the fibers into separate longitudinal fascicles each of which contains fibers of similar physiological type from a single root which has a definite peripheral distribution. These fascicles retain their individuality and amongst them is a very limited amount of neuropil containing the synapses between the collaterals and terminals of the root fibers and the dendrites of the neurones of the second order whose cell bodies lie in the underlying

gray substance. Some of the root fibers leave their respective fascicles to enter the bundles of arcuate fibers, probably to terminate in the tegmental areas of the same and the opposite sides. Golgi sections show many free arborizations of arcuate fibers ending in the motor tegmentum and especially in the motor nuclei of the cranial nerves. The number of such fibers is small in the young larvae and most of the root fibers evidently end in relation to the neurones of the stratum griseum of the adjacent parts of the sensory region on the same side of the oblongata.

In the adult the general pattern of the root fibers is similar to that of the larva, the same arrangement of longitudinal tracts being present; but in this case the related areas of neuropil are larger, the number of medially directed root fibers in the arcuate tracts is greater, and there are some other important changes, though all features of the adult appear to have been derived directly from the larval relations here described, which I regard as primitive for Amphibia.

THE TERMINAL NUCLEI AND SENSORY TRACTS RELATED TO THE SENSORY ROOTS OF THE CRANIAL NERVES

In all of the more highly differentiated brains the various sensory roots of the cranial nerves terminate in special gray centers, each of which serves as the end-nucleus of a special functional system. As we have seen above, the arrangement of the sensory roots of larval *Amblystoma* as they enter the oblongata conforms to the typical vertebrate pattern and each root maintains its individuality to its ultimate terminus. But the neurones of the second order which form the terminal nuclei of these root fibers are practically all found in the primitive relation as a central gray layer, within which in ordinary histological preparations there is very little evidence of any specialization. But if this central gray substance were functionally equipotential, as it appears to be, the question would arise, how could the central analysis of sensory stimuli be effected?

To answer this question I have examined a large number of Golgi preparations of larval and adult *Amblystoma* brains with results which are somewhat surprising. Previous to the exact

analysis of the peripheral nerve roots and their central courses the impression made upon me by these preparations was that the apparatus for the central analysis and correlation of sensory stimuli from the cranial nerves was almost as simple and undifferentiated as in the spinal cord; but after more careful study it became evident that the dendrites of the neurones of the gray substance are distributed throughout the white substance in accordance with a very definite functional pattern, some of the details of which can now be stated.

The dendrites of the neurones of the gray substance, as in the spinal cord, may ramify widely throughout the white layer, and in very few cases have I observed all of the dendritic arborizations of any neurone entering into functional connection with the terminals of a single sensory root. In other words, the medulla oblongata of larval *Amblystoma* contains no groups of neurones which may be regarded as specific terminal nuclei for the various sensory nerve roots described in the preceding section. On the other hand, almost every one of the hundreds of neurones of the sensory region of the oblongata which are satisfactorily impregnated in my preparations sends its dendrites outward to effect synaptic relation with the termini of two or more distinct sensory roots. Moreover, these dendrites are not spread out at random among the fascicles of root fibers and other tracts, but the arrangement seems to conform to a definite functional pattern in each case. I have by no means completed the analysis of these relations, but the observations already made give a fairly exact general idea of the plan of organization of these brains.

The neurones associated with the fasciculus solitarius (visceral sensory system) appear to be more sharply circumscribed than those related to any other functional system, and some of these appear to be functionally related to no root fibers other than those represented in this fasciculus (fig. 40, *nuc.f.sol.*). Nevertheless nearly all of the neurones related to the fasciculus solitarius send some of their dendrites also into the region of distribution of the spinal V root or some other distinct system (see fig. 38).

The region dorsally of the fasciculus solitarius and spinal V root receives the VIII and lateral line roots, and the gray substance

contiguous to these roots appears to possess a certain loosely organized physiological unity, for the neurones of this gray substance tend to spread out within the stratum album of this region only and very often to reach practically all parts of it. This portion of the oblongata (including both the white and the gray layers) may, therefore, properly be called the area acustico-lateralis. Nevertheless individual neurones are sometimes seen to send dendrites into definite restricted parts only of this area and other dendrites into the spinal V root or even into the underlying tegmentum (figs. 27, 28, 29, 35).

The dendrites of the neurones of the area acustico-lateralis which are related to the roots of the lateral line nerves may pass directly outward into the white layer, but more commonly they run for considerable distances in the marginal zone of the gray substance and give thick contorted branches into the white layer, where they end in dense tufts among special tracts, each neurone thus usually effecting synaptic relations with one or more roots of both the lateralis VII and the lateralis X and perhaps also with the VIII roots (figs. 34, 36) and with correlation tract *a* or *b*. Other forms of these endings are seen in figures 27, 32, 35. The most important synapses are probably effected in the marginal zone of the white substance related to the respective fiber tracts of the white substance adjacent, for here are found the densest tufts of dendritic arborizations.

The axones of these sensory neurones of the second order are directed downward and inward in the marginal zone of the gray substance. As arcuate fibers, after decussation in the ventral commissure, some effect various forms of reflex connection within the oblongata and a larger number enter the bulbar lemniscus, or tractus octavo-tectalis et thalamicus (*lm.* of the figures). This tract is comparable with the fasciculus lateralis of the oblongata of fishes and in part with the lateral lemniscus of mammals. It is a compact bundle of fibers of medium size, heavily myelinated in the adult, which ascends to the midbrain and largely terminates by free arborizations in the stratum album of the tectum mesencephali. A smaller number of its fibers, however, continue forward to terminate in the thalamus.

The details of the bulbar and spinal connections of the neurones of the area acustico-lateralis I have not been able to determine. Throughout the length of the oblongata there is a tract of fibers dorsally of the bulbar lemniscus arising in part from the motor tegmentum of the same and the opposite side which increases rapidly in size as it passes from in front caudad. This is the tractus bulbo-spinalis (figs. 10 to 17, *tr.b.sp.*), and it is probable that descending fibers from the area acustico-lateralis and other sensory centers of the oblongata are also associated with this tract for bulbo-spinal reflexes.

The neurones related with the VIII roots are of the same type as the preceding, and I have seen no case of a neurone related to endings of the VIII nerve which does not also send a dendrite into the lateral line roots or into the spinal V root or both. No evidence of specially differentiated cochlear or vestibular nuclei has been found in the larva, but in the adult such differentiated cells are manifest. The peculiar relations of Mauthner's cell will be considered beyond (see p. 379).

A typical neurone of the sensory V nucleus under the eminentia trigemini at the level of the superficial origin of the root is seen in figure 24. The chief dendrite spreads out in the distribution area of collaterals from the root fibers (cf. figs. 49 and 50), but other dendrites enter the areas of the VIII root and of the tractus spino-bulbaris et tectalis. Still other dendrites enter the marginal zone of gray substance, where they may engage termini of arcuate fibers. In figure 23 an incomplete impregnation of three similar neurones is seen, the axon being directed downward into the ventral commissure. In figure 25 is another group of these neurones, whose dendrites reach the sensory V fibers, the ascending secondary visceral tract, the tractus spino-bulbaris et tectalis and also the underlying tegmentum. An exactly similar neurone from a level slightly farther rostrad is seen in figure 22. In this case a dendrite also enters the field of VIII root fibers. Still farther forward under the cerebellum (fig. 21) are two neurones of the eminentia cerebelli ventralis which are apparently of the same type, the dendrites ending chiefly among the fibers of the sensory V root and the tractus spino-bulbaris et tectalis. Again

a short distance farther forward, at the level where the tractus spino-cerebellaris separates from the tractus spino-bulbaris et tectalis (fig. 20), are neurones which clearly belong to the cerebellum and which closely resemble those last described. The dendrites of these neurones engage terminals of the sensory root fibers of the cranial nerves and of the tractus spino-bulbaris and spino-cerebellaris systems; they also reach into the motor tegmentum. The axones are more slender than those of the neurones last described and apparently form part of the tractus cerebello-tegmentalis system. The nature of the terminals of the tractus spino-cerebellaris in this region is shown in figures 19 and 47. The more lateral neurone shown in figure 31 is a type frequently observed in relation with the spinal V root. This neurone should probably be regarded as belonging to the substantia gelatinosa Rolandi, for its chief dendrites end in relation with fibers of the spinal V root. Yet other dendrites reach the VIII roots, the ascending secondary visceral tract and the motor tegmentum. Figures 26 and 29 illustrate two tangential neurones whose dendrites also effect synaptic relations with the sensory V root fibers. These highly specialized neurones will be considered later in connection with the tegmentum (p. 379). In the vagus region there are interesting neurones whose dendrites connect with both the spinal V root and the fasciculus solitarius, which will be described immediately.

I have no observations on the neurones of the second order related to the prefacial fasciculus solitarius, but in the vagus region our Golgi preparations illustrate many of these elements. Figure 40 shows an impregnation of several types of neurones. The most dorsal of these belongs in the lobus visceralis dorso-medially of the fasciculus solitarius and its dendrites arborize chiefly within this fasciculus. Numerous axones of these elements are impregnated. They are seen to take two divergent courses. Some pass ventro-laterally to enter the ascending secondary visceral tract of the same side; others pass ventro-medially to enter the ventral commissure, beyond which they cannot be separately followed.

Other neurones in this preparation lie immediately ventrally of the fasciculus solitarius. Their dendrites are directed chiefly

ventro-laterally into relation with the spinal V root, some, however, reaching the fasciculus solitarius, the VIII root, the ascending secondary visceral tract, the spino-bulbar tract and the motor tegmentum. The predominant physiological characteristic of these neurones is probably determined by their connection with the spinal V root; i.e., they are comparable with those of the mammalian substantia gelatinosa Rolandi. Nevertheless it is evident that they are not exclusively devoted to this function. A single neurone of this sort is seen also in figure 41 and two others in figure 39, the latter figure illustrating also the mode of termination of the visceral sensory vagus root fibers. Figure 38 shows a single neurone, some of whose dendrites arborize in the fasciculus solitarius and some in the spinal V root. The axon is apparently directed into the ascending secondary visceral tract. In several cases I have seen similar axones dividing, one branch entering the ascending secondary visceral tract of the same side, the other crossing in the ventral commissure to the other side.

Figure 39 on the left side shows the dendrite of a neurone lying among those which we have above compared with the substantia gelatinosa Rolandi entering the fasciculus solitarius. The axon of this neurone can be followed across the ventral commissure and into the tractus bulbo-tectalis of the opposite side. In Golgi, Cajal and Weigert preparations of both larvae and adults the tractus bulbo-tectalis is seen to be made up of similar fibers from the most ventral part of the ventral commissure. This tract can be followed caudad as far as the bulbar lemniscus (see fig. 16), viz., as far as the caudal end of the area acustico-lateralis. Reading the sections forward from this level, the tractus bulbo-tectalis increases in size and follows closely the ventral border of the bulbar lemniscus into the tectum mesencephali (figs. 4 to 16), its fibers being smaller and in the adult less heavily myelinated than those of the lemniscus. It enters the tectum superficially of the lemniscus (figs. 4 to 6) and in the caudal part of the midbrain it ascends rapidly to a position dorsally of the lemniscus, where its fibers immediately terminate in free arborizations among the dendrites of the nucleus posterior tecti, which are directed forward to meet them. My evidence as to the location of the cells from

which these fibers originate is very incomplete, but so far as it goes it suggests that they come from the group of neurones which I have designated above (p. 372) as substantia gelatinosa Rolandi, and chiefly in the vagus region. This tractus bulbo-tectalis would therefore be, in part at least, the trigeminal lemniscus, with probably a certain gustatory or general visceral element also represented.

The ascending secondary visceral tract (*tr.v.a.*) is apparently the amphibian equivalent of the teleostean secondary gustatory tract (secondary vagus bundle of Mayser). It terminates in an area of neuropil in the isthmus region which has already been mentioned (p. 365) as the probable representative of the superior secondary gustatory nucleus of teleosts (Herrick '05), or "Ridenknoten" of Mayser ('81); see figures 5, 6, 52, *tr.v.a.* Dendrites of neurones which apparently belong in the basal part of the body of the cerebellum enter this neuropil, and this is the same region in which I have seen termini of the tractus mammillo-cerebellaris of *Necturus* ('14, fig. 19). This neuropil is conspicuously developed in adult *Amblystoma* and unmedullated strands are directed forward and downward from it toward the hypothalamus at a deeper level than the tractus mammillo-cerebellaris. These may contain the tertiary gustatory tract, as I have found it in fishes ('05).

In figure 39 the most dorsal dendrite shown on each side arises from a neurone whose cell body lies dorso-laterally of the fasciculus solitarius, and in figure 44 two similar neurones are impregnated. The dendrites of these neurones arborize chiefly among the fibers of the correlation tract *b* of Kingsbury and the axones are directed medialward toward the ventral commissure. These neurones would, therefore, seem to be adapted to transmit impulses from the ventral correlation tract of the area acustico-lateralis to the opposite side of the oblongata. Their further connections are unknown.

The connections of the two longitudinal tracts *a* and *b* are likewise unknown. Fibers are seen to pass between both of them and various fasciculi of root fibers and also the system of arcuate fibers. Kingsbury ('95, p. 175) states that in *Necturus* arcuate

fibers enter the ventral tract *b*, chiefly between the levels of the IX and X nerves, most of the bundles of arcuate fibers turning cephalad within the tract, but some caudad. These tracts do not extend beyond the limits of the area acustico-lateralis. At their rostral ends they converge and the united tract reaches the tip of the auricular lobe. Both of these tracts, particularly tract *a*, are bordered laterally by a dense superficial neuropil in the limiting zone of the white substance. From time to time fibers leave the tracts to enter this outer plexiform layer and there come into relation with dense dendritic arborizations of the neurones of the area acustico-lateralis. It appears probable that the fibers of these tracts are chiefly derived from the opposite side of the oblongata, either as root fibers or secondary fibers. Each neurone of the area acustico-lateralis would, in this case, engage root fibers of its own side and also either root fibers or secondary fibers from the area acustico-lateralis of the opposite side.

There remain to be considered two important tracts concerned with the correlation of sensory impressions from the spinal cord to the oblongata, viz., the fasciculus dorso-lateralis of the spinal cord and the tractus spino-bulbaris system.

In the upper levels of the spinal cord there are bundles of fibers dorsally and medially of the spinal V root (fig. 18, *f.d.l.*) which appear to conduct impulses from the sensory area of the spinal cord into the oblongata. This fasciculus dorso-lateralis of the spinal cord can be followed forward into the vagus region as far as the caudal end of the area acustico-lateralis, its rostral end lying wholly ventrally of this area. Further details regarding connections of these fibers have not been determined. They may extend much farther forward.

Ventrally of the fasciculus dorso-lateralis and spinal V root in the caudal end of the oblongata is another ascending fasciculus (fig. 17, *tr.sp.t.*), which in figures 12 to 17 is designated simply tractus spino-tectalis. In reality this is a complex system of fibers arising from unknown cells of the spinal cord and terminating at intervals throughout the length of the oblongata, cerebellum, midbrain and thalamus. Briefly, it is the spinal lemniscus and spino-cerebellar system.

From the lower end of the oblongata to the V roots these fibers are all assembled into a compact fascicle ventrally of the spinal V root (figs. 9 to 17). Golgi sections show terminals from this tract turning inward to end throughout the motor tegmentum as far forward as the eminentia subcerebellaris tegmenti and especially among the dendrites of the motor VII and motor V nuclei. This is the tractus spino-bulbaris. In the vicinity of the V roots, in both the larva and the adult, special fascicles leave this tract to terminate in the chief sensory V nucleus under the eminentia trigemini, some of these fascicles ascending in company with the ascending sensory V root fibers far forward under the cerebellum. These fibers constitute a tractus spino-bulbaris trigemini (figs. 7, 8, *tr.sp.b.*), and probably serve to bring spinal cutaneous nervous impulses forward into relation with those from the skin of the head in the eminentia trigemini.

Shortly below the level of the V roots the mixed tract divides into a lateral spinal lemniscus portion (figs. 10, *tr.sp.t.*) and a more medial spino-cerebellar portion (*tr.sp.cb.*). Under the body of the cerebellum the latter portion (figs. 7, *tr.sp.cb.*) turns abruptly dorsalward and divides into two parts. Some of these fibers end in the body of the cerebellum of the same side and a larger number of them continue directly upward to enter the commissura cerebelli and finally to terminate in the cerebellum of the opposite side.

In my examination of the cerebellum of *Necturus* ('14, p. 7) I described, in addition to the tract just described, a tractus spino-cerebellaris dorsalis. More complete study of these tracts has not yielded a clear separation of the latter tract, the fibers so described, if present at all, being so mingled with other systems as to make their separate identification uncertain. In the aberrant teleost, *Mormyrus*, Stendell ('14) has recently described a spino- or bulbo-cerebellar tract which arises at the caudal end of the oblongata from the terminal nucleus of the fasciculus dorso-lateralis of the spinal cord. This large tract passes for its entire length through the oblongata dorsally of the spinal V root, and may be the equivalent of the tractus spino-cerebellaris dorsalis which I provisionally identified in *Necturus*.

The tractus spino-tectalis, after separation from the tractus spino-bulbaris and tractus spino-cerebellaris (fig. 7, *tr.sp.t.*) crosses from the lateral to the medial side of the tractus spino-cerebellaris, takes up its position medially of the bulbar lemniscus (*lm.*) and then ascends into the tectum mesencephali (figs. 4 to 6). It can be followed as several small compact bundles of fibers throughout the length of the midbrain, and here most of its fibers end by free arborizations which spread widely through the lateral part of the tectum. Some of these fibers, however, continue farther forward to end in the thalamus.

The fibers of the spino-bulbar, spino-cerebellar, spino-tectal and spino-thalamic tracts appear to form a single system of closely related elements. They are only incompletely separated even in the rostral end of the oblongata under the cerebellum, and collaterals of the spino-tectalis fibers have in several cases been seen to enter the cerebellum (figs. 19 and 47).

Summary. The secondary sensory tracts of the oblongata of larval *Amblystoma* may be summarized as follows: The neurones of the second order in the sensory region are generally related to two or more different roots, so that few of the secondary tract fibers conduct sensory impulses derived from a single type of peripheral sense organ. Nevertheless the chief tracts are comparable in a general way with the corresponding tracts of mammals and probably each has a single dominant physiological function.

There are reflex connections between the sensory centers and the motor nuclei of the oblongata and cord, but the details of these pathways have not been determined. The ascending secondary tracts are more clearly defined. There is an important ascending system in the dorso-lateral fasciculus of the spinal cord which ends in the caudal end of the oblongata, and a second system farther ventrally which extends the entire length of the brain stem as far forward as the thalamus. This spinal lemniscus contributes fibers to the motor centers of the oblongata (tractus spino-bulbaris), to the eminentia trigemini (tractus spino-bulbaris trigemini), to the cerebellum (tractus spino-cerebellaris), to the midbrain (tractus spino-tectalis), and to the thalamus (tractus spino-thalamicus). From the area acustico-lateralis of the oblon-

gata the fibers of the bulbar lemniscus arise (tractus octavo-tectalis et thalamicus). These fibers decussate in the ventral commissure and terminate in the tectum mesencephali and thalamus. Accompanying the latter tract ventrally and laterally is a smaller fascicle which I have termed the tractus bulbo-tectalis; this arises (in part at least) from the secondary trigeminal neurones of the vagus region, these same neurones being also connected with the visceral sensory root fibers of the fasciculus solitarius. These fibers cross in the ventral commissure and terminate in the nucleus posterior tecti of the midbrain. Their physiological significance is not wholly clear, but they seem to share the functions of a trigeminal lemniscus and a secondary visceral tract. Between the tract last mentioned and the spinal V root is a smaller bundle of non-myelinated fibers which are derived from the neurones of the visceral sensory lobe of the same side and which terminate in a small secondary visceral nucleus in the isthmus, this tract being clearly comparable with the secondary vagus bundle of Mayser, or the ascending secondary gustatory tract of Herrick, in teleosts.

THE MOTOR NUCLEI AND TRACTS

The cells of the ventral gray column of the spinal cord which give rise to ventral root fibers in larval *Salamandra* have been figured by Van Gehuchten ('97, pl. 29, fig. 1). Neurones of the same type are seen in our figures 17 and 18, *col.v.*, though I have not seen root fibers arising from these cells.

The approximate boundaries of the motor nuclei of the V, VI, VII, IX and X cranial nerves are indicated in figure 1. It is difficult to determine the precise limits of these nuclei, for their neurones are mingled with those of the motor tegmentum and in none of our Golgi preparations are the axones of the motor roots impregnated. Neurones which probably belong to the nucleus ambiguus (*nuc.amb.*) are shown in figures 37, 39 and 41. Dendrites of these cells spread out widely in the tegmentum and also in the secondary visceral tract (*tr.v.a.*). Mingled with these are other similar neurones whose axones are directed ventrad into the ventral funiculi of the same side and of the opposite side. The

tractus bulbo-spinalis (figs. 10 to 18, *tr.b.s.*) probably arises from these cells. Dendrites of the large tangential neurones of the VII, IX and X regions cross the meson in the ventral commissure and their axones are in some cases seen to enter the fasciculus longitudinalis medialis, some ascending and some descending. Other neurones of the system of the motor tegmentum are seen in figures 25, 27, 29, 30, 31, 33, 37, 38, 39, 41 and 42.

Laterally of the motor nuclei of the VII, IX and X cranial nerves is a tegmental area containing relatively small neurones in the stratum griseum, whose slender dendrites spread widely throughout the white substance and also in the marginal zone of the gray substance among the arcuate fibers (figs. 27, 33). These neurones, whose axones are generally directed downward toward the ventral commissure with numerous collaterals, probably are of the same type as those of the formatio reticularis of higher brains.

The VI nerve emerges from the brain by several delicate rootlets some distance caudad of the motor VII roots, as stated by Coghill ('02, p. 213). I have seen no Golgi impregnations of this nucleus, but in Cajal preparations of the larvae its neurones do not appear to differ from the small cells of the surrounding central gray. In the adult, however, these neurones are considerably enlarged. This nucleus closely surrounds the fasciculus longitudinalis medialis, a special fascicle of which breaks up among its cells. This fascicle is derived chiefly from the area acustico-lateralis of the same side, and some of its fibers can be followed caudad as far as the motor IX nucleus.

The limits of the motor VII nucleus are fairly clear in Cajal preparations, but within and around this nucleus are tegmental neurones; see figures 11, 12, 13, 27, 30, 33. The motor V nucleus, on the other hand, lies farther lateral than the motor VII and is more clearly separated from the nucleus motorius tegmenti. Medially of the motor V nucleus is a tegmental eminence which is greatly enlarged farther forward as the eminentia subcerebellaris tegmenti. Into this eminence are discharged ascending tracts from the spinal cord (tractus spino-bulbaris) and also important descending tracts from the cerebrum (figs. 4 to 7), such as the tractus tecto-bulbaris (*tr.t.b.*), tractus thalamo-bulbaris (*tr.th.b.*),

and fasciculus lateralis telencephali (lateral forebrain bundle, *tr.lat.t.*). This subcerebellar eminence is separated from the motor tegmentum in front of the isthmus by a sharp sulcus (fig. 2) which runs obliquely forward and downward from the recessus posterior mesencephali. In younger stages this sulcus is shallower and more nearly transverse, and in the adult it is almost entirely obliterated. The eminence itself appears to have resulted from a cellular proliferation in the area of discharge of the prosencephalic tracts mentioned above and to serve as a distribution area for these descending impulses to the motor tegmentum of the lower levels of the oblongata.

The most highly specialized neurones of the urodele oblongata are sparsely scattered in the marginal zone of the gray substance with their chief dendrites spread widely among the arcuate fibers which pass through this zone. These cells I term collectively for descriptive purposes the tangential neurones. These tangential cells are correlation neurones of various sorts and are probably derived, in part at least, from the "commissural cells" of Coghill ('14, fig. 27, *Com.*) in very young larvae. The development and connections of these neurones merit further study.

One pair of these tangential neurones is greatly enlarged in most species of fishes and amphibians and has long been known as the cells of Mauthner. The position of Mauthner's cell in the half grown larva is seen in figures 12 and 54, and figure 53 shows a projection of the cell and its chief processes upon the transverse plane in a 12 mm. larva. The portion of the cell here shown reaches through seven sections each 5μ in thickness and thus extends about 35μ in a cephalo-caudal direction. Only the larger processes of the cell can be followed in this preparation, but these reach practically the entire cross section of the stratum album at this level. The axones of Mauthner's cells decussate and descend in the fasciculus longitudinalis medialis in the manner usually described. In this specimen they are heavily myelinated. Myelinated fibers are also seen in the fasciculus longitudinalis medialis and in some other tracts of the white substance.

In the young larva the dorsal dendrite of Mauthner's cell spreads throughout the distribution area of all of the lateral line

roots (fig. 53), processes arising near the cell body extend outward among the entering fibers of the VIII root, some above and some below the fasciculus solitarius (see fig. 12 of an older larva) and big dendrites extend medialward among the arcuate fibers and ventralward to reach all of the tegmental tracts. A distinct fascicle of descending fibers from the fasciculus longitudinalis medialis turns laterally from that tract and terminates about the medial dendrites and body of Mauthner's cell (fig. 54). Mauthner's cells and fibers are also present in adult *Amblystoma*.

The relations above described are for the most part confirmatory of the observations made by Beccari ('07) upon other species of *Urodela*. The literature of Mauthner's cells has been fully reviewed by Beccari. I merely call attention to the fact that we have here an apparatus, found in those aquatic *Ichthyopsida* which swim by means of the trunk and tail musculature, which may be stimulated from the periphery through the VIII and lateral lines nerves or centrally by ascending tracts from the spinal cord (tractus spino-bulbaris system), by descending tracts from the midbrain (tractus tecto-bulbaris and fasciculus longitudinalis medialis) or locally from the motor tegmentum. The general cutaneous and visceral systems do not appear to participate in this innervation. The axon extends downward throughout the spinal cord and is known to discharge into the somatic motor centers.

Summary. The nuclei of the motor roots of the cranial nerves can be identified, though they are incompletely separated from the general motor tegmentum. A greatly enlarged portion of the latter region is developed under the cerebellum (eminentia sub-cerebellaris tegmenti) for the reception of descending cerebral tracts, and from the whole motor tegmentum of the oblongata fibers of the tractus bulbo-spinalis system arise. In the marginal zone of the gray substance are found large tangential neurones whose dendrites connect with the sensory field and tegmentum and whose axones enter the fasciculus longitudinalis medialis and tractus bulbo-spinalis. These are the most highly differentiated correlation neurones of the oblongata. Two neurones of this system are greatly enlarged and are known as Mauthner's cells.

GENERAL SUMMARY AND DISCUSSION

A summary of the more important central relations of the sensory roots of the cranial nerves of *Amblystoma* will be found on page 366 and a summary of their chief secondary connections on page 376 and on page 380 is a summary of the motor tracts and centers. In the following paragraphs some of the more general results of this study are presented and discussed.

The external form and histological structure of the medulla oblongata of half grown larvae of *Amblystoma* resemble closely those of the adults of the lower urodeles, such as *Necturus*. An accurate analysis of the functional components of the cranial nerves of older larvae and adults of *Amblystoma* has been published by Coghill ('02), who is now publishing also a series of correlated physiological and anatomical studies on much younger larvae. The observations here presented may serve to connect these researches by illustrating the functional pattern of the medulla oblongata at an intermediate stage of development. The author also hopes to use them as a point of departure for further studies on the development and functional connections of the cerebellum, midbrain and thalamus of urodeles.

The factors operating in either the ontogenetic or the phylogenetic differentiation of the correlation centers of the brain cannot be profitably investigated without a precise knowledge of the peripheral relations of each functional system represented in these centers and of the interrelations of these systems at every step in the progress of the nervous impulses transmitted by them during the course of normal functional activity.

In the present analysis of the central courses of the cranial nerve roots it has been found that the individual fibers of each sensory root of the V, VII, VIII, IX and X nerves bifurcate immediately upon entering the medulla oblongata into ascending and descending branches. The ascending and descending fibers of each root are united into distinct fascicles which retain their individuality from the superficial origin of the root upward and downward respectively throughout nearly the entire length of the oblongata. All of the somatic sensory root fibers divide in this way and many, if not most, of the visceral sensory fibers also

do so. The general arrangement of these fascicles of root fibers is shown in figure 3.

From this it follows that the white substance (*stratum album*) of the sensory region of the oblongata from the level of the spinal V tract dorsalward contains a series of longitudinal fascicles of root fibers, each of which is a functional unit and whose fibers are distributed peripherally to a particular species of sense organs. These fascicles of root fibers occupy the entire *stratum album* of this part of the oblongata, save for the presence of two additional bundles of correlation fibers (the tracts *a* and *b* of Kingsbury) and for a variable amount of a superficial neuropil in the marginal zone of the white substance. This arrangement is preserved throughout the length of the acustico-lateral area of the oblongata, whose limits are indicated in figure 3 by the distribution area of the VIII and lateralis root fibers.

The neurones of the gray layer (*stratum griseum*) of the sensory region of the oblongata send their dendrites outward to arborize among the terminals of these root fibers and the synaptic connections here effected are made in accordance with a definite functional pattern; but this pattern is far less simple than that of the root fibers and there are few groups of neurones of the gray substance which can be regarded as the specific or pure terminal nuclei of particular functional systems of sensory roots. Most of these neurones effect synaptic connection within the white layer with more than one physiological type of root fibers, from which it follows that the functions of primary receptor centers and correlation centers are in these cases united to some extent within the individual neurones of the second order. The white substance of the medulla oblongata of these urodeles contains, in addition to the motor and sensory root fibers, a large number of correlation fibers, and the longer systems of these fibers are arranged in definite correlation tracts.

From the relations described in the preceding paragraph it is evident that these correlation tracts are intermediate in physiological type between those of the spinal cord of these larvae and those of the oblongata of mammals. In the spinal cord of larval urodeles there is little evidence of localization of function in the

sensori-motor apparatus, but each of the larger correlation neurones (and many of the peripheral motor neurones also) seems to effect synaptic relations with the entire stratum album, to wit, with sensory root fibers of all kinds and also with all of the long correlation tracts of the cord. In the mammalian medulla oblongata, on the other hand, each functional component of the various sensory roots of the cranial nerves terminates in a specially differentiated end-nucleus, from which neurones of the second order conduct the nerve impulses of their respective sensory types either directly to the motor centers through the reticular formation or by long tracts to higher correlation centers. These secondary tracts transmit unmixed specific sensory excitations of the same functional type as the roots with which they are functionally related. Finally, in the oblongata of urodele larvae we find a third type of correlation neurones. The peripheral neurones of the sensory roots of the cranial nerves exhibit a specificity of function which appears to be as precisely localized as that of the corresponding mammalian nerves and to show essentially the same arrangement of nerve components in the cranial roots. The regional localization of the end-stations of these various functional types of root fibers is tolerably precise and clearly defined. But the sensory neurones of the second order are not arranged in similarly circumscribed nuclei related respectively to these definite end-stations of the peripheral neurones. On the other hand, practically all of the neurones of the adjacent gray substance send their dendrites into at least two and often into several different end-stations, so that the same secondary neurone may be, and apparently habitually must be, capable of excitation by two or more diverse end-organs. Thus, a single neurone of the oblongata may effect synaptic connection with glossopharyngeal fibers from taste buds and also with trigeminal fibers of tactile sensibility (fig. 38), with root fibers from the V and also from the VIII cranial nerves (fig. 31), or with fibers of the tactile sense coming in from the head by way of the V cranial nerve and with fibers of the tractus spino-bulbaris conveying tactile impulses from the trunk region (figs. 21, 22, 23, 24, 25). Each sensory neurone of the second order in the oblongata is, accordingly, at the same time a correlation neu-

rone and the apparatus for the sensory analysis of peripheral stimuli is greatly simplified as compared with the mammalian condition, though more perfect than that in the spinal cord of urodeles.

Nevertheless a certain degree of functional localization in the gray substance is evident. The fasciculus solitarius is the most distinctly separate bundle of root fibers and the related neurones of the lobus visceralis are evidently under the exclusive or dominant physiological influence of this system of root fibers.

In the somatic sensory area the secondary neurones related with the V root fibers are tolerably distinct, though in all cases related also with other systems, particularly the VIII root, the secondary visceral tract and the underlying tegmentum. The neurones related with the VIII and lateral line roots form a fairly distinct group, the area acustico-lateralis, the more dorsal members of this system of neurones being related exclusively with the various lateral line roots (always with more than one of these roots) and the more ventral neurones having more diversified connections. In the adult the functional localization of specific terminal nuclei within the area acustico-lateralis is far more completely elaborated than in the larva.

Though the physiological segregation of the secondary neurones into specific sensory centers is incomplete in these larvae and few of the tracts of the second order carry pure sensory impulses derived from a single functional system of peripheral root fibers, yet in most cases some one of these peripheral systems evidently dominates the pathway. In the further evolutionary history of these secondary pathways the dominant system in each case appears to have persisted to the exclusion of the subordinate connections, and the functions of correlation are thus transferred from the primary receptor centers to the reticular formation for the simple bulbar reactions and to the higher cerebral centers for the more complex reactions. We are, accordingly, able to recognize the mammalian equivalents of most of the amphibian correlation tracts, though the homology in all of these cases is incomplete owing to the imperfect segregation of the amphibian primary terminal nuclei of the cranial nerve roots.

The gray substance of the oblongata shows progressively more clearly defined areas of functionally distinct neurones as we pass from the spinal end forward. And the anatomical arrangement suggests that the capacity for diversified reactions is greater in those parts of the body innervated from the anterior end of the oblongata. Physiological observation, of course, shows that this is the case.

In *Amblystoma* of different ages various forms of reflex connections are now known and these can be arranged in a graded series, starting from the primitive reflex apparatus of the very early swimming larva, which may be conceived of as suggesting some of the steps in the phylogenetic evolution of the mechanism of correlation as found in the brains of higher vertebrates.

Coghill has shown ('14) that in the simple swimming reflex of very early developmental stages of *Amblystoma* there is very little evidence of specificity of function even in the peripheral sensory neurones. Each of these neurones (the transitory giant cells of Rohon and Beard in the spinal cord) may connect peripherally with both the skin and the myotom, the cutaneous and the muscle sense innervation cooperating to maintain the swimming reflex. These transitory cells are in later developmental stages replaced by those of the spinal ganglia, in which the neurones concerned with cutaneous and deep sensibility are probably distinct as in higher vertebrates.

In the spinal cord of the half grown larva, however, the neurones of the gray substance show very imperfect functional localization, each of these neurones apparently being physiologically related to all types of peripheral sensory nerves. Here also any stimulus whatever on the trunk will evoke a simple swimming movement.

In the medulla oblongata of these half grown larvae the peripheral neurones show a high degree of functional specificity, and the central neurones of the second order tend to be grouped around these special sensory roots. But the functional localization of these secondary neurones is not complete, each neurone having a dominant relation to some particular peripheral root but subsidiary connections also with other functionally distinct roots.

Thus each primary bulbar center reached by terminals of root fibers is to some extent also a correlation center and the analysis of function in the reactions is still incomplete.

In the mammals the functional differentiation of the primary bulbar centers is complete and the functions of correlation are transferred to higher cerebral centers. Simpler total reactions of the more primitive sort are, however, still provided for in the formatio reticularis of these higher brains.

The examination of the series of types of reflex pattern now known in *Amblystoma* suggests the dominance of the integrative function of the vertebrate central nervous system from its earliest phylogenetic phases. The simplest responses to external stimulation, such as the avoiding reaction and the swimming reflex of very young larvae (Coghill), are simple total reactions involving the coordinated action of large masses of body musculature. Within such a unitary reflex system, which necessarily requires the orderly participation of extensive regions of the nervous system, more refined special movements may be differentiated, and this involves the segregation of particular functional areas within the originally unitary system under the influence of the progressive differentiation of the receptor and effector apparatuses.

Throughout this process of differentiation the correlation centers, as they become more individualized from the primary unitary system, are pushed farther and farther back from the periphery; but at no stage in the process is the primitive dominance of the integrative function of the system as a whole lost. The preservation of the functional integrity of the individual during the process of differentiation of its parts is the most important function of the higher correlation centers, as Sherrington has so graphically shown.

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DESCRIPTION OF FIGURES

All figures are drawn from preparations of larvae of *Amblystoma tigrinum* and, except where otherwise expressly stated, each figure is drawn from a single preparation and is not a composite from several sections. All of the Golgi sections figured are taken from specimens of larvae of about the age of the one modelled or older. In the figures of Golgi preparations the boundary between the gray and the white layers is indicated by a heavy broken line.

REFERENCE LETTERS

- | | |
|--|---|
| <i>a.ac.</i> , area acustico-lateralis | <i>M.f.</i> , Mauthner's fiber |
| <i>Aq.</i> , aqueductus Sylvii | <i>n.IV.</i> , nervus trochlearis |
| <i>b.p.</i> , bipolar neurones of lateralis VII ganglion | <i>n.IX.</i> , nervus glossopharyngeus |
| <i>br.conj.</i> , brachium conjunctivum | <i>n.m.t.</i> , nucleus motorius tegmenti |
| <i>cb.</i> , cerebellum | <i>n.sp.1.</i> , first spinal nerve |
| <i>c.cb.</i> , corpus cerebelli | <i>nuc.amb.</i> , nucleus ambiguus |
| <i>col.v.</i> , columna ventralis grisea | <i>nuc.f.sol.</i> , nucleus of fasciculus solitarius |
| <i>com.cb.</i> , commissura cerebelli (medullated component) | <i>nuc.mes.V.</i> , nucleus mesencephalicus V |
| <i>com.cb.l.</i> , commissura cerebelli (lateral unmedullated component) | <i>nuc.IX.m.</i> , nucleus motorius IX |
| <i>com.i.s.</i> , commissura infima Halleri (somatic component) | <i>nuc.p.t.</i> , nucleus posterior tecti |
| <i>com.i.v.</i> , commissura infima Halleri (visceral component) | <i>nuc.V.m.</i> , nucleus motorius V |
| <i>dec.veli.</i> , decussatio veli | <i>nuc.VI.m.</i> , nucleus motorius VI |
| <i>em.cb.v.</i> , eminentia cerebellaris ventralis | <i>nuc.VII.m.</i> , nucleus motorius VII |
| <i>em.s.t.</i> , eminentia subcerebellaris tegmenti | <i>nuc.X.m.</i> , nucleus motorius X |
| <i>em.V.</i> , eminentia trigemini | <i>n.V.</i> , nervus trigemini |
| <i>f.d.l.</i> , fasciculus dorso-lateralis | <i>n.VI.</i> , nervus abducens |
| <i>f.l.m.</i> , fasciculus longitudinalis medialis | <i>n.VII+VIII.</i> , nervi facialis et acusticus |
| <i>f.l.m.VI.</i> , tract from area acustico-lateralis to VI nucleus | <i>pl.c.</i> , plexus chorioideus |
| <i>f.sol.</i> , fasciculus solitarius | <i>r.IX.mot.</i> , radix motorius IX |
| <i>g.V.</i> , ganglion of V nerve | <i>r.l.</i> , recessus lateralis rhombencephali |
| <i>hyth.</i> , hypothalamus | <i>r.l.l.VII.</i> , radix lateralis facialis |
| <i>lm.</i> , bulbar lemniscus (from area acustico-lateralis) | <i>r.l.l.X.</i> , radix lateralis vagi |
| <i>lob.au.</i> , lobus auricularis rhombencephali | <i>r.p.m.</i> , recessus posterior mesencephali |
| <i>lob.vis.</i> , lobus visceralis | <i>r.V.</i> , sensory root of the V nerve |
| <i>M.c.</i> , Mauthner's cell | <i>r.V.mes.</i> , radix mesencephalica V |
| <i>mes.</i> , mesencephalon | <i>r.V.mes.d.</i> , descending fibers of radix mesencephalica V |
| | <i>r.V.mot.</i> , radix motorius V |
| | <i>r.VI.</i> , radix abducentis |
| | <i>r.VII.l.l.</i> , radix lateralis facialis |
| | <i>r.VII.l.l.d.</i> , radix lateralis facialis dorsalis |
| | <i>r.VII.l.l.m.</i> , radix lateralis facialis medius |

- r.VII.l.l.v.*, radix lateralis facialis ventralis
r.VII.mot., radix motorius facialis
r.VII.v.s., visceral sensory root of the facialis
r.VIII., radix nervi acustici
r.VIII.d., radix dorsalis acustici
r.VIII.v., radix ventralis acustici
r.v.I.sp., first ventral spinal root
r.X., radix nervi vagi
r.X.l.l.d., radix lateralis vagi dorsalis
r.X.l.l.v., radix lateralis vagi ventralis
r.X.m., radix motorius vagi
r.X.s.s., somatic sensory (general cutaneous) root of the vagus
r.X.v.s., visceral sensory root of the vagus
r.X.2 to r.X.7, second to seventh roots of the vagus
s.7.R., substantia gelatinosa Rolandi
s.l., sulcus limitans
tect., tectum mesencephali
tegm., tegmentum
tr.a., dorsal longitudinal tract of area acustica
tr.b., ventral longitudinal tract of area acustica
tr.b.sp., tractus bulbo-spinalis
tr.b.t., tractus bulbo-tectalis
tr.cb.teg., tractus cerebello-tegmentalis
tr.lat.t., fasciculus lateralis telencephali (lateral forebrain bundle)
tr.m.cb., tractus mammillo-cerebellaris
tr.sp.cb., tractus spino-cerebellaris
tr.sp.b., tractus spino-bulbaris
tr.sp.t., tractus spino-tectalis
tr.t.b., tractus tecto-bulbaris
tr.th.b., tractus thalamo-bulbaris
tr.v.a., tractus visceralis ascendens (secondary gustatory tract)
t.v.q., taenia ventriculi quarti
u.p., unipolar neurones of lateralis VII ganglion

Fig. 1 Dorsal view of a wax model of the medulla oblongata of a 38 mm. larva of *Amblystoma tigrinum*. $\times 42$. The plexus chorioideus has been removed. On the right side of the model the positions of the underlying motor nuclei of the cranial nerves are indicated, as projected upon the ventricular surface. On the scales above and below the figure are given the serial numbers of the transverse sections upon which the model is based, the sections being 15μ thick. Drawings from 15 of these sections, which were prepared by the silver reduction method of Ramón y Cajal, are shown in figures 4 to 18. The method used produces considerable shrinkage and distortion of the form relations, especially in the cerebrum. In the medulla oblongata the tissue is of firmer texture and the distortion is less. Comparison with other specimens of larvae of the same age prepared by various other methods indicates that in this model the only important distortion of the natural form relations is manifested by a slight exaggeration of the ventricular sulci and by a partial collapse of the thin-walled caudal end of the tectum mesencephali near the mid-dorsal line into the recessus posterior mesencephali (cf. figs. 5 and 6).

Fig. 2 View of the medial surface of the same model after division in the sagittal plane. $\times 42$.

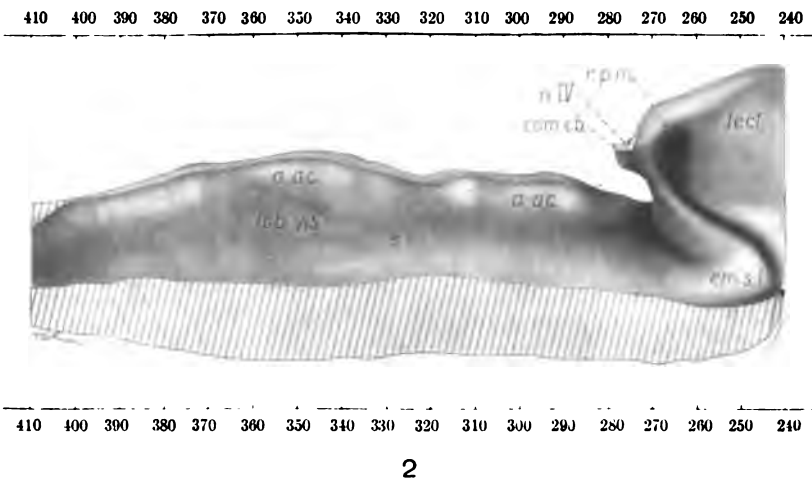
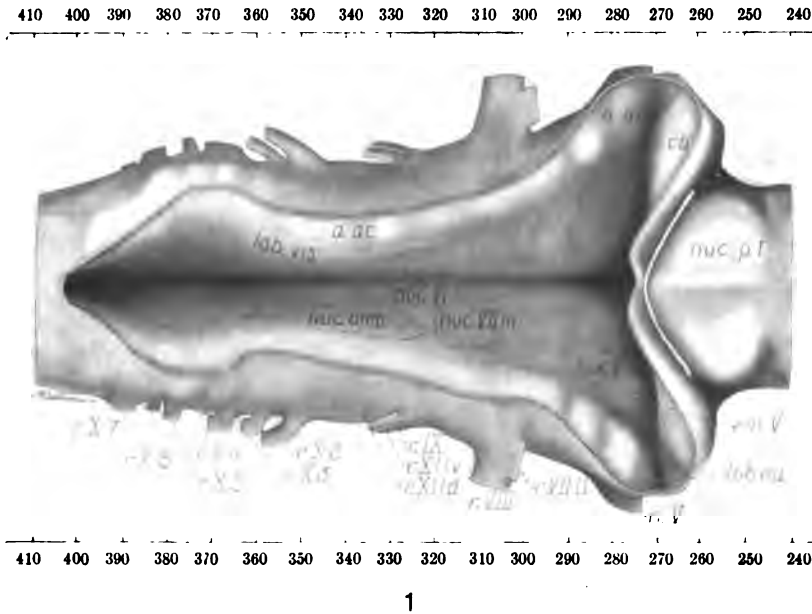
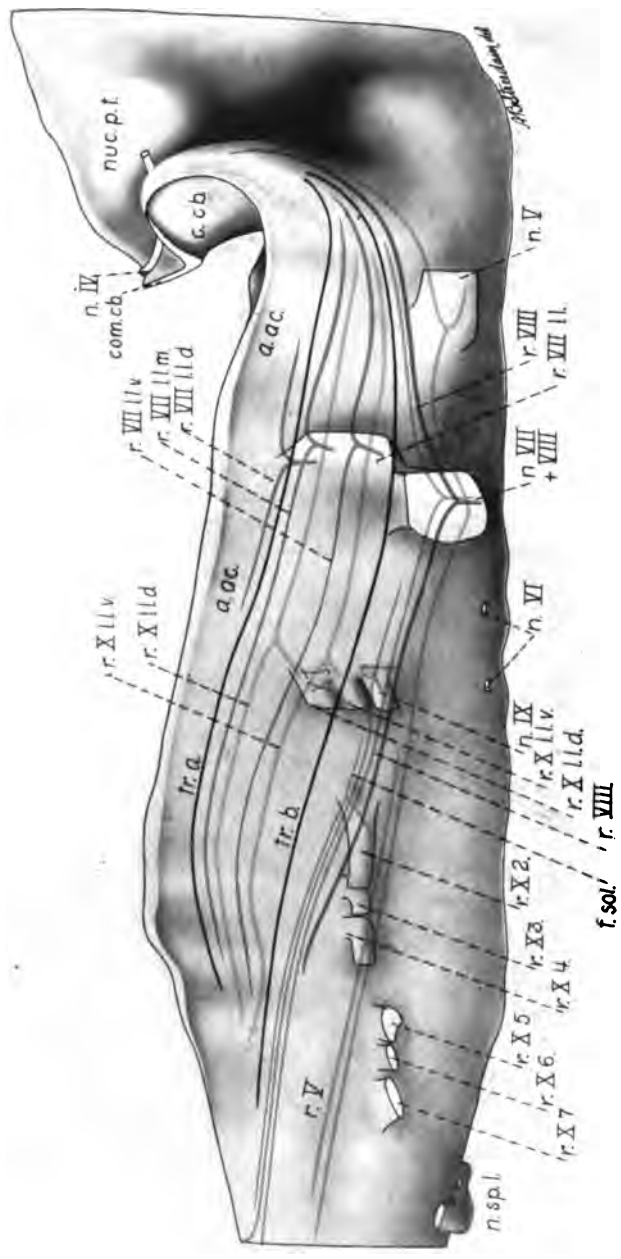


Fig. 3 View of the lateral surface of the same model shown in figures 1 and 2. $\times 62$. The superficial origins of the various cranial nerve roots are shown in the perspective drawing, and in colors the courses of the individual root fibers of the various sensory components. The general cutaneous roots of the V and X nerves are shown in yellow, the VIII root in blue, the visceral sensory roots of the VII, IX and X nerves (fasciculus solitarius or fasciculus communis component) in red, the dorsal and ventral lateral line X roots in green, and the three lateral line VII roots in brown. The dorsal and ventral correlation tracts of the area acustico-lateralis (tracts *a* and *b* of Kingsbury) are shown in black. The mesencephalic V root is omitted; for the relations of this root see figure 57.

410 400 390 380 370 360 350 340 330 320 310 300 290 280 270 260 250 240



410 400 390 380 370 360 350 340 330 320 310 300 290 280 270 260 250 240

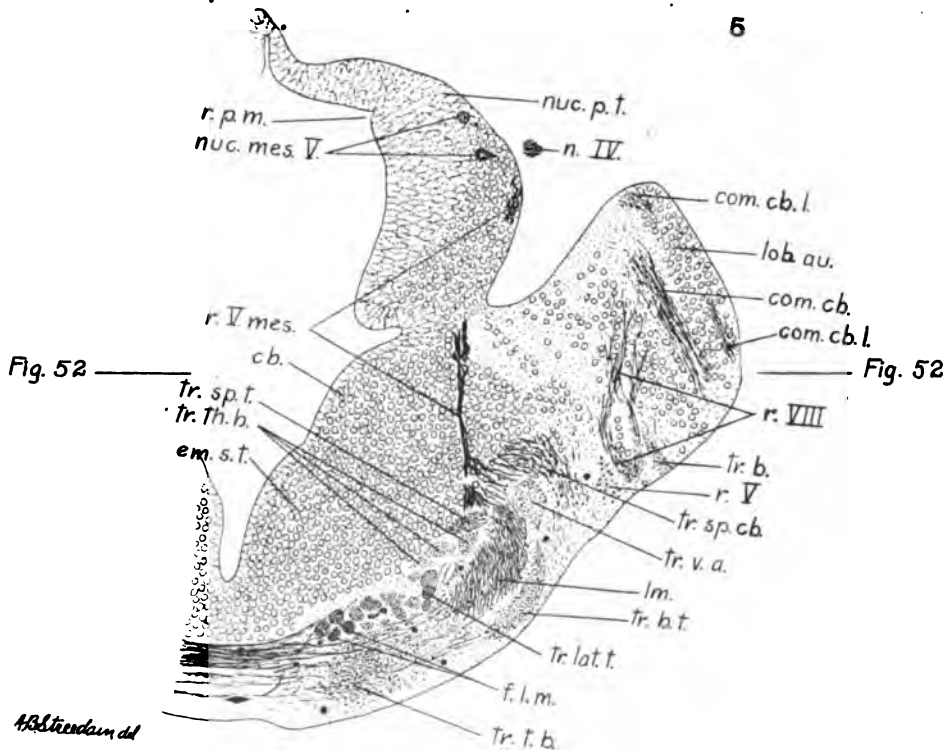
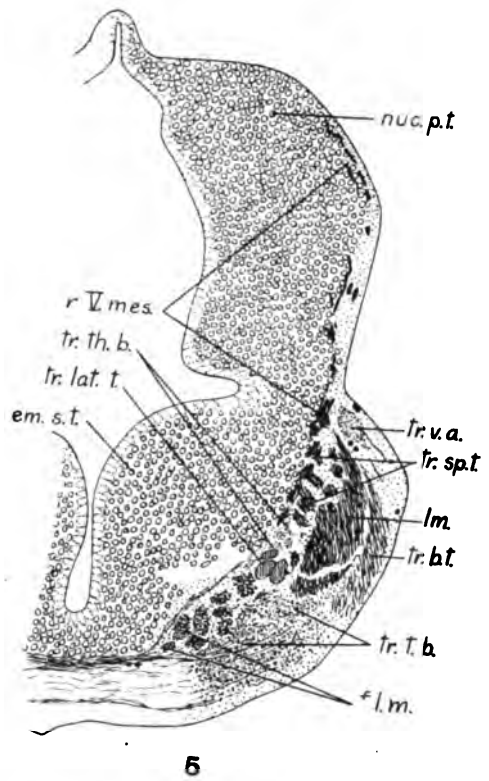
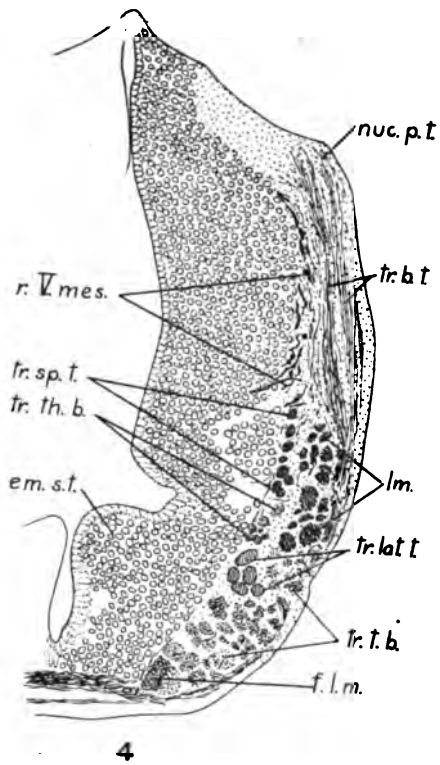
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Figs. 4 to 18 Fifteen transverse sections through the brain of a 38 mm. larva of *Amblystoma tigrinum*, prepared by the silver reduction method of Ramón y Cajal. These drawings were made from the same series of sections used in the construction of the model shown in figures 1 to 3. In the following descriptions the serial number of each section figured is given. By comparison with the scales accompanying figures 1 to 3 the exact place of the section figured in the model can be determined, the sections being 15μ thick. The drawings were outlined with the Edinger projection apparatus, and in projection the image was reversed so as to permit a direct comparison of the drawing with the appearance of the preparation under the compound microscope. The mirror-image of these drawings would, therefore, give the correct relations. In the model, figures 1 to 3, the natural relations of right and left sides are preserved, so that figures 4 to 18, though apparently representing the right side of the brain, should be compared with the left side of the model. The two sides of the specimen figured are in all essential respects similar, save for slight differences in the arrangement of the vagus roots at their superficial origins. The nuclei of the cells of the stratum griseum are drawn as clear circles, those of the scattered cells of the stratum album as black discs. These latter nuclei, which are of uncertain significance, stain darker in these preparations than those of the stratum griseum. Some of the larger neurones of the motor nuclei of the cranial nerves and of the marginal zone of the gray layer are drawn with shaded nuclei and clear cytoplasm. The topographical relations of the fiber tracts of the stratum album are drawn as faithfully as possible, but the texture of these tracts is schematically indicated to facilitate following each tract through the series of sections.

Fig. 4 Section 245, through the caudal part of the midbrain immediately in front of the nucleus posterior tecti. The cell bodies of this nucleus lie farther caudad; the dendrites of these cells extend forward into the neuropil marked *nuc.p.t.*, which also receives terminals of the tractus bulbo-tectalis (*tr.b.t.*). The section also includes the rostral end of the eminentia subcerebellaris tegmenti (*em.s.t.*).

Fig. 5 Section 255, through the nucleus posterior tecti (*nuc.p.t.*) above and the pedunculus cerebri below, immediately rostral to the auricular lobes, cutting through the eminentia subcerebellaris tegmenti (*em.s.t.*) at its widest part.

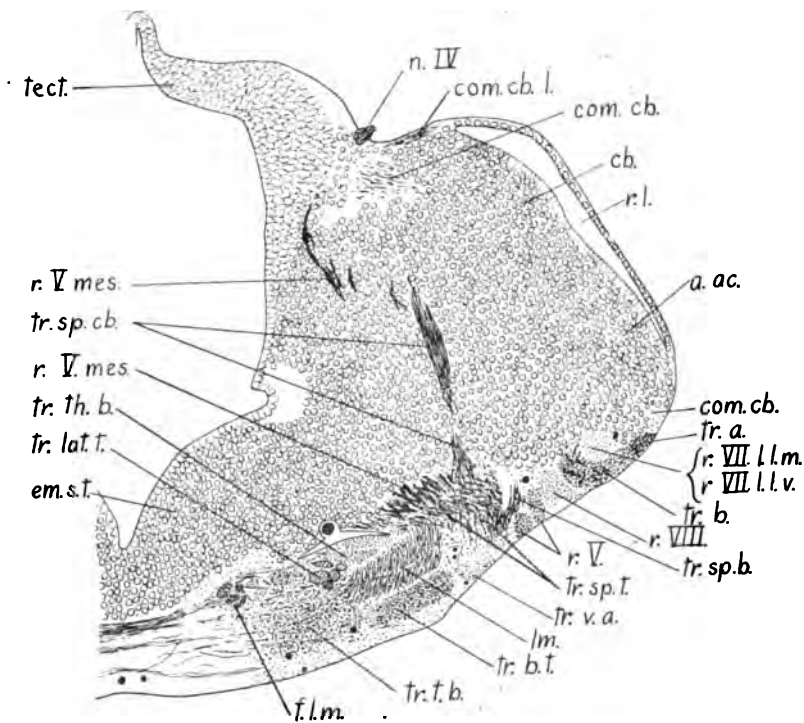
Fig. 6 Section 260, through the rostral end of the auricular lobe (*lob. au.*), including the terminals of the ascending roots of the V and VIII cranial nerves. The plane of section of figure 52 is indicated by guide lines.



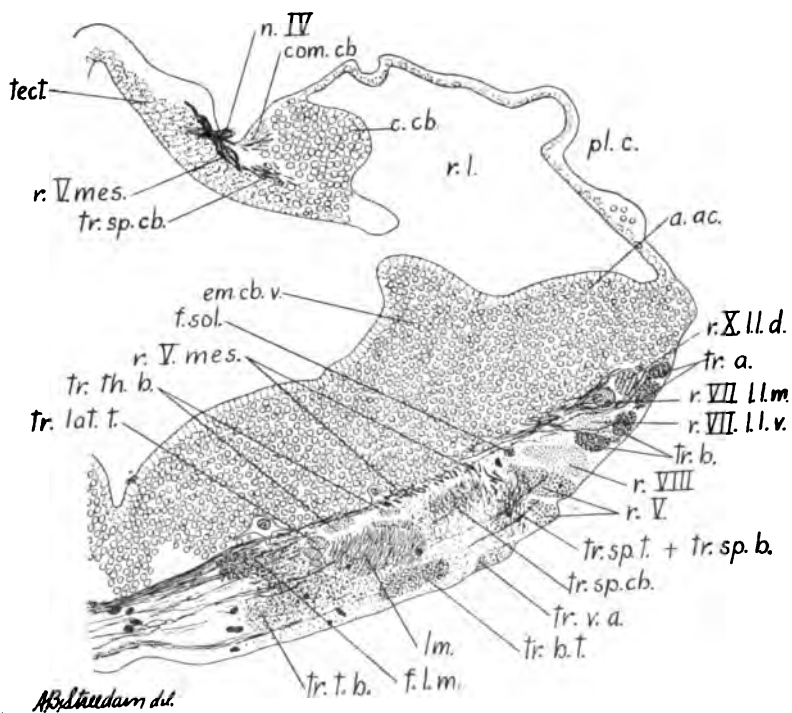
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Fig. 7 Section 265, through the auricular lobe, the body of the cerebellum and the rostral end of the recessus lateralis (*r.l.*). In the medial wall of the recessus lateralis are two eminences separated by a shallow ependymal groove. The dorsal eminence is the body of the cerebellum (*cb.*), which has been differentiated in the rostral wall of the recessus lateralis of earlier embryonic stages; the ventral eminence is the rostral end of the area acustico-lateralis (*a.ac.*), which at this age has been partially incorporated into the cerebellum. The eminentia subcerebellaris tegmenti (*em.s.t.*) has merged with the motor tegmentum of the oblongata. The arrangement of the fiber tracts in the stratum album of the ventral part of the section has assumed the form characteristic of the oblongata in general.

Fig. 8 Section 270, immediately caudad of the junction of the body of the cerebellum (*c.cb.*) with the eminentia cerebellaris ventralis (*em.cb.v.*), the latter lying medially of the area acustico-lateralis (*a.ac.*). The caudal end of the tectum mesencephali is included in the section and the decussatio veli lies a few sections farther caudad.



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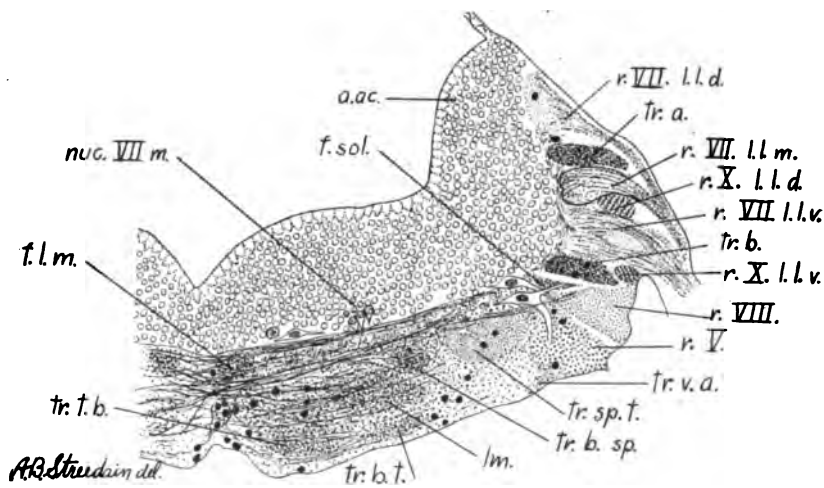
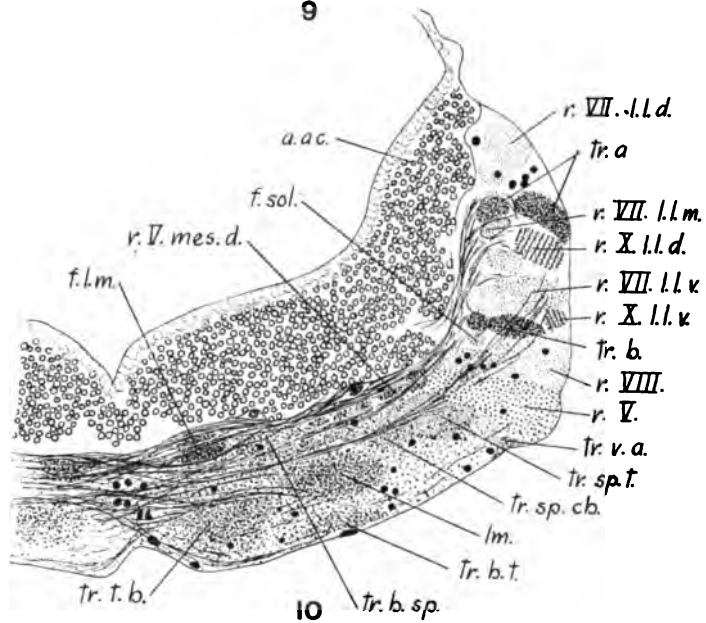
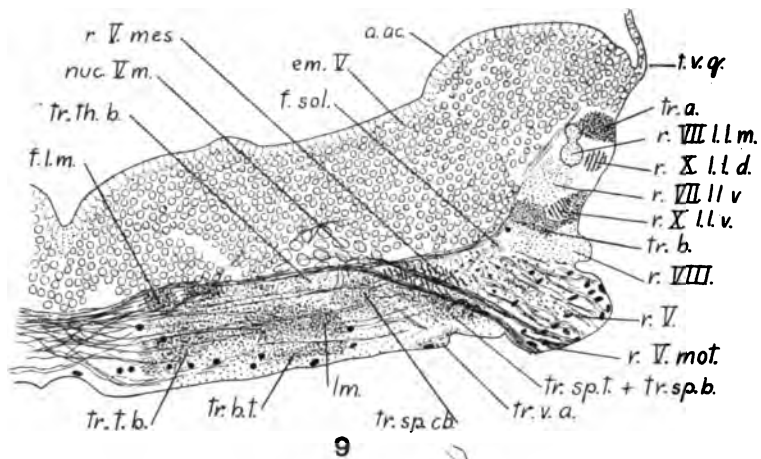
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Fig. 9 Section 280, through the superficial origin of the V cranial nerve and the motor V nucleus. The eminentia trigemini (*em. V.*) is formed partly by the motor V nucleus, but chiefly by the sensory V nucleus. This eminence is more prominent in some specimens than here shown.

Fig. 10 Section 295, between the V and VII roots.

Fig. 11 Section 301, at the level of entrance of the three lateral line VII roots, and including the rostral end of the motor VII nucleus.

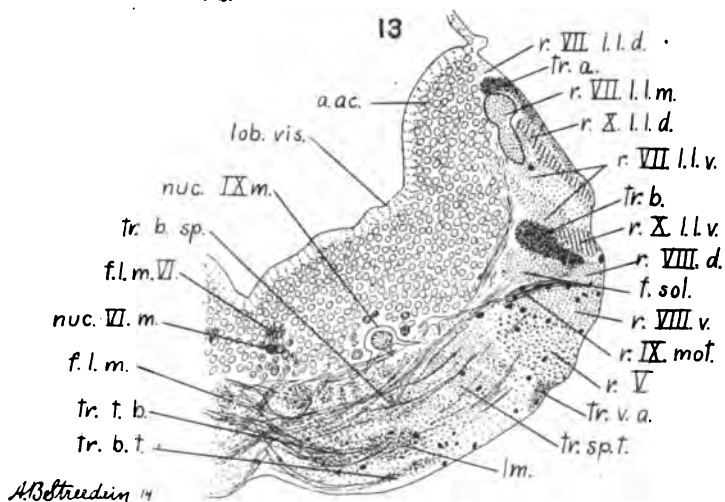
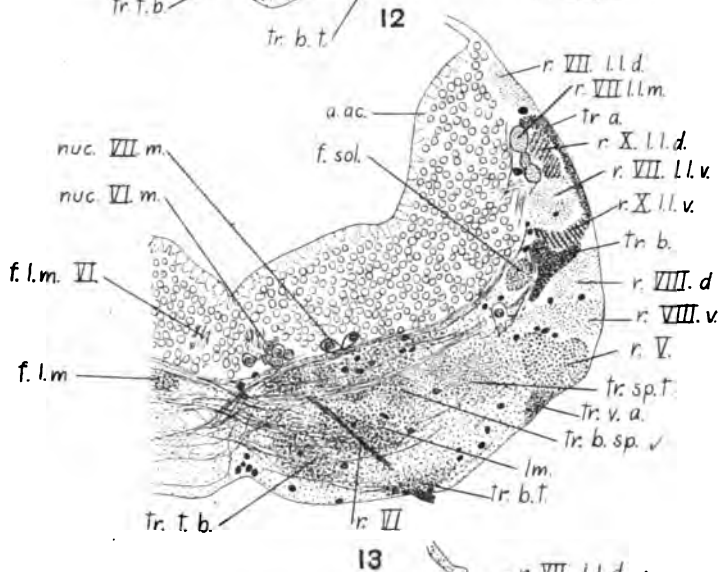
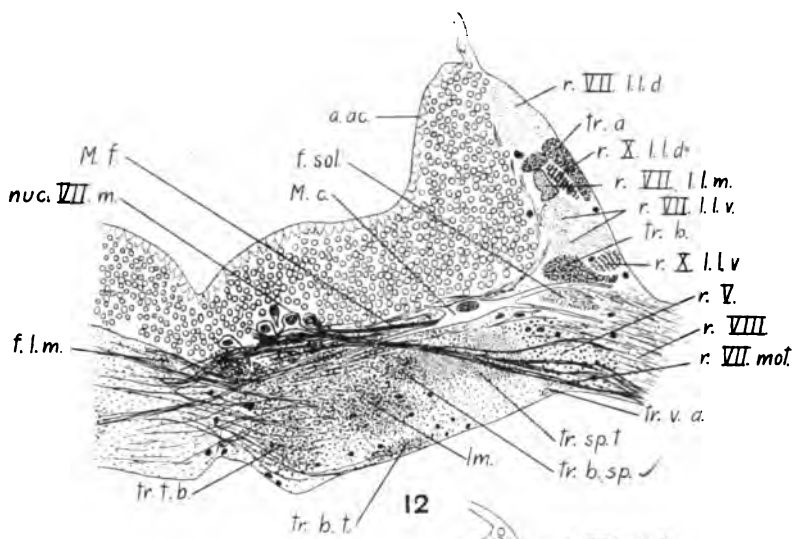


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Fig. 12 Section 307, at the level of the VIII and motor VII roots. The visceral sensory (communis) root of the VII nerve enters the fasciculus solitarius immediately rostrally of this level. The greater part of Mauthner's cell lies in the plane of this section. Two lateral dendrites of this cell are figured, one above and one below the fasciculus solitarius, the ventral one of these dendrites having been sketched in from the adjacent section 308. The cells of the motor VII nucleus (*nuc. VII.m.*) were also entered upon this drawing from section 308.

Fig. 13 Section 320, between the VIII and IX roots, at the level of the rostral rootlet of the VI nerve.

Fig. 14 Section 327, immediately rostrally of the IX and lateral line X roots, including the motor root and nucleus of the IX nerve.



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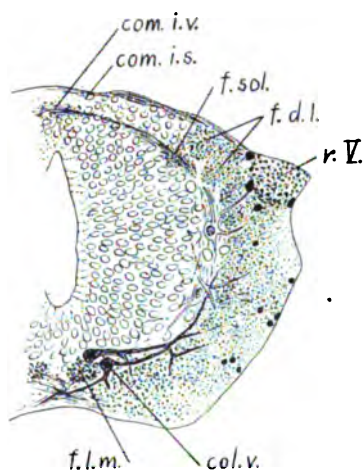
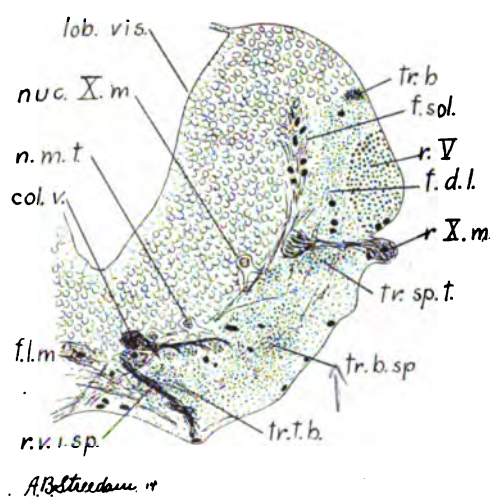
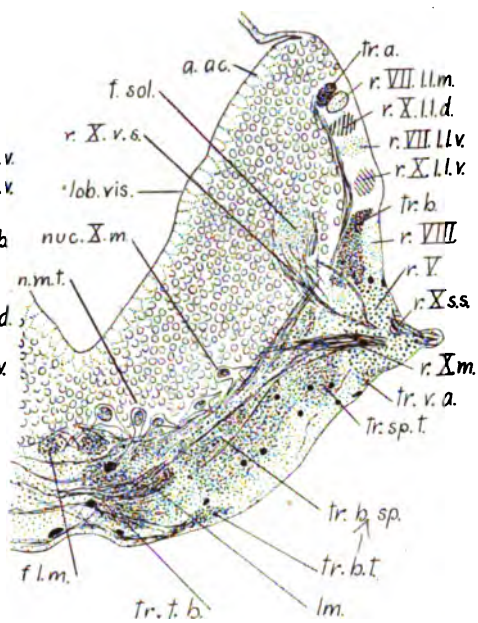
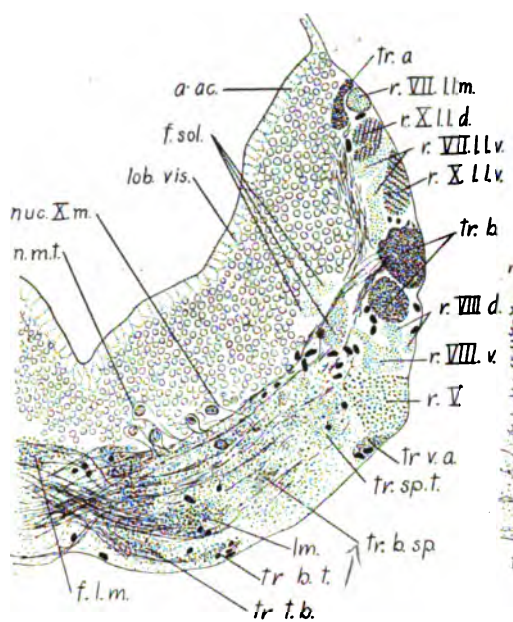
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Fig. 15 Section 340, between the lateralis and the second roots of the vagus.

Fig. 16 Section 365, at the level of the fourth root of the vagus. The somatic sensory, visceral sensory and motor components of this root are distinguishable.

Fig. 17 Section 390, at the level of the seventh root of the vagus and the most rostral rootlets of the first spinal nerve. In this figure and figure 18 the neurones which are supposed to give rise to ventral root fibers (*col.v.*) are darkly shaded, the other neurones of the marginal zone of the gray substance are drawn in outline only.

Fig. 18 Section 410, at the level of the commissura infima Halleri. The somatic component of this commissure (*com.i.s.*) is seen to be related to the region of the spinal V root (*r.V.*) and fasciculus dorso-lateralis of the spinal cord (*f.d.l.*). The visceral component (*com.i.v.*) is related to the fasciculus solitarius (*f.sol.*) and to the commissural nucleus of Cajal in the mid-dorsal region. The strong lateral eminence produced by the spinal V root has been exaggerated by shrinkage of the specimen during preparation.



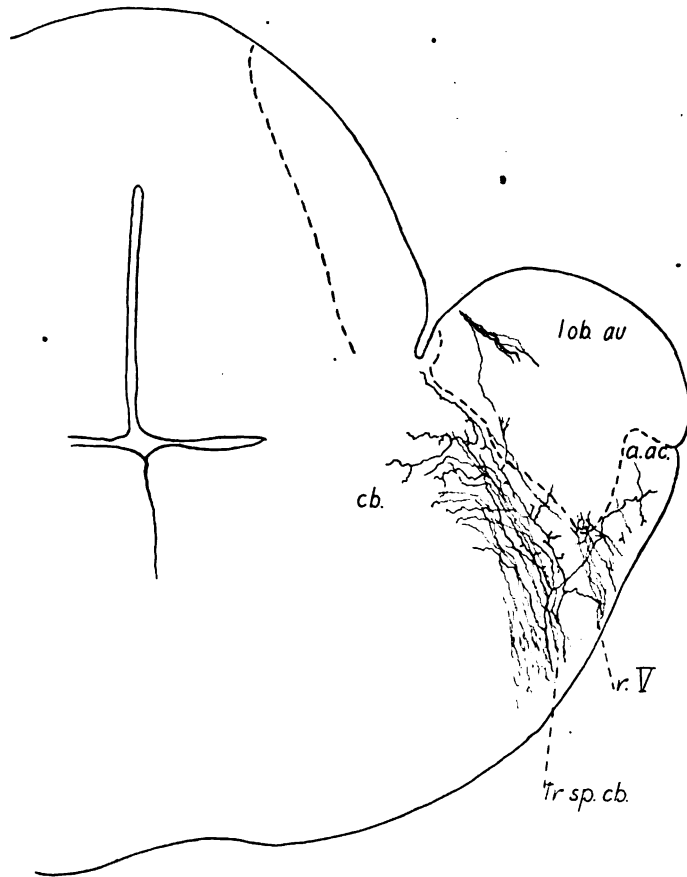


Fig. 19 Transverse section through the rostral end of the auricular lobe (*lob. au.*), illustrating collaterals from the tractus spino-tectalis and tractus spino-cerebellaris ending in the cerebellum. Some of these fibers are also seen to enter the rostral end of the area acustico-lateralis (*a.ac.*), among terminals of root fibers from cranial nerves. Most of the latter fibers here impregnated probably come from the trigeminus (*r.V.*). The plane of section is very slightly caudad of that of figure 6. Golgi method. $\times 100$.

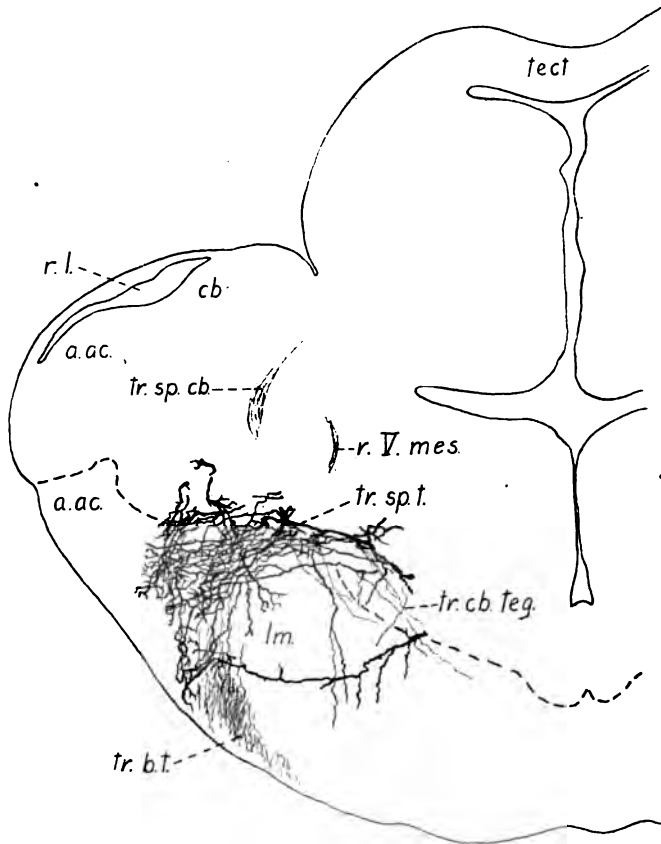


Fig. 20 Transverse section through the rostral end of the auricular lobe and recessus lateralis (*r.l.*). Fibers of the tractus spino-cerebellaris (*tr.sp.cb.*) and mesencephalic V root (*r.V.mes.*) are seen passing upward through the body of the cerebellum to enter the decussatio veli. The tractus bulbo-tectalis (*tr.b.t.*) ascends to enter the tectum laterally of the bulbar lemniscus (*lm.*), which is not impregnated. Dendrites of cerebellar neurones are seen entering the stratum album of the area acustico-lateralis and the region of the tractus spino-tectalis, which is not impregnated; cf. figures 6 and 19. Axones of these neurones (*tr.cb.teg.*) are passing downward toward the ventral commissure. Golgi method. $\times 100$.

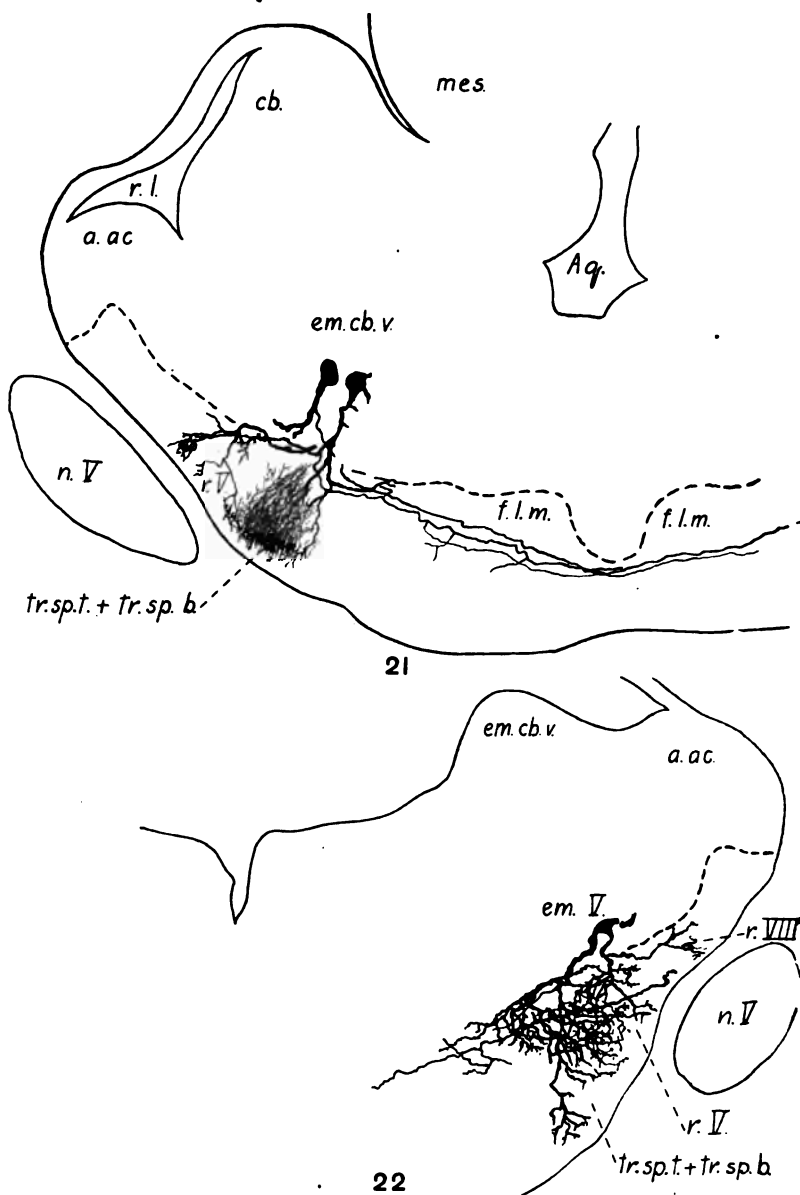


Fig. 21 Transverse section between the levels shown in figures 7 and 8, including two neurones of the eminentia cerebellaris ventralis (*em.cb.v.*). This region, which appears to be a highly modified portion of the rostral end of the terminal nucleus of the ascending sensory root fibers of the trigeminus (general cutaneous component), is incorporated into the cerebellum of the adult, in much the same way as is the rostral end of the area acustico-lateralis which receives root fibers of the VIII and lateralis roots. Golgi method. $\times 100$.

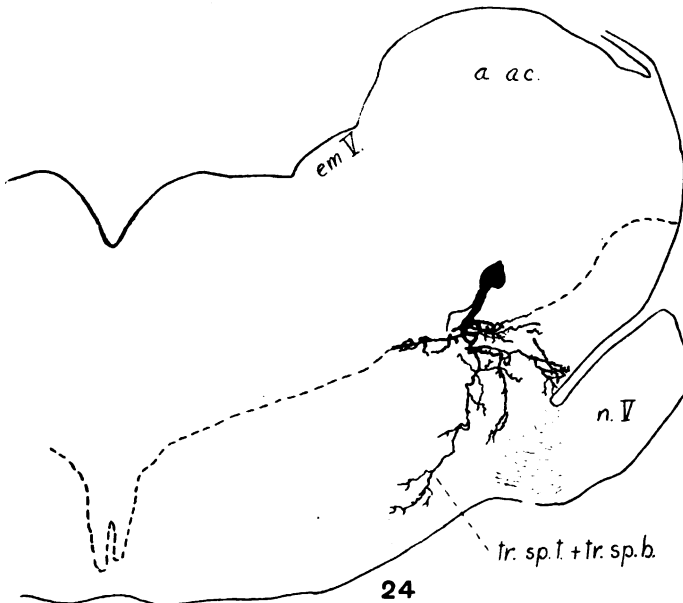
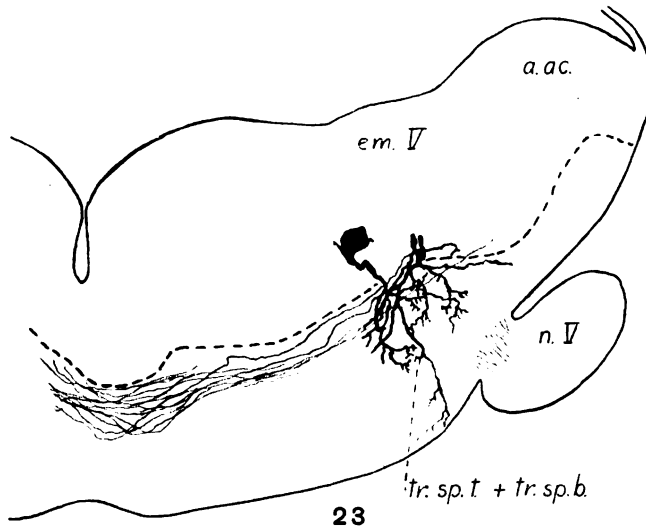
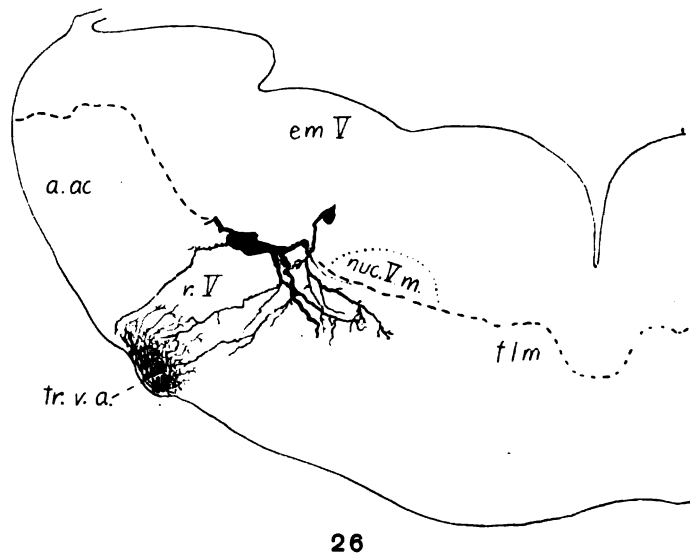
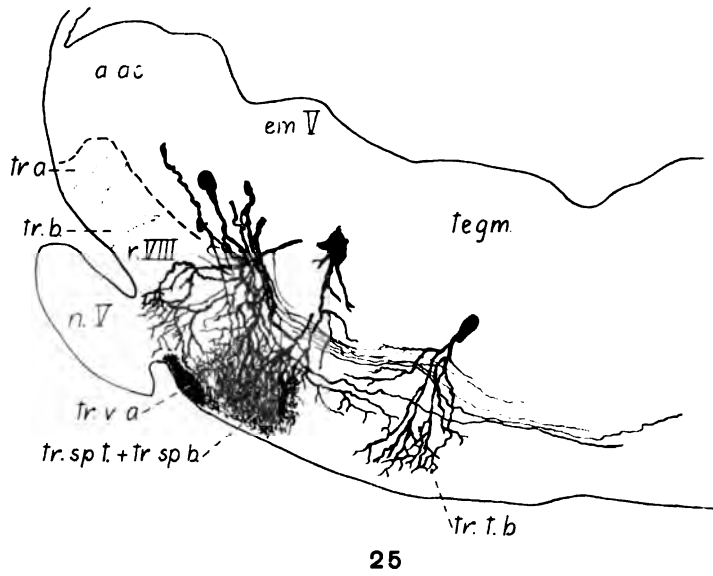
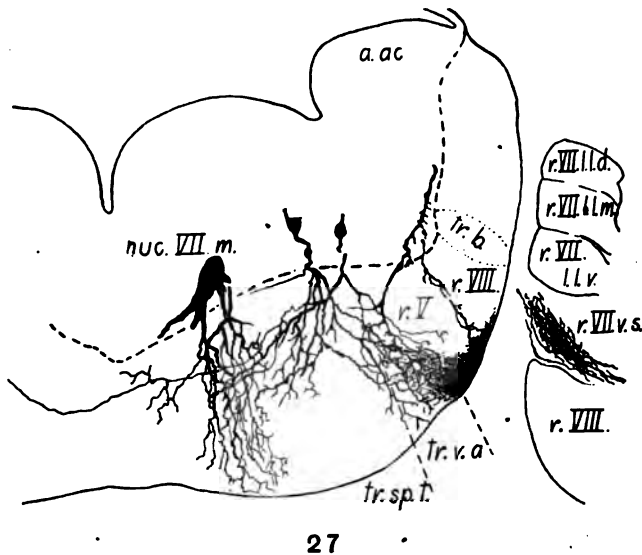


Fig. 22 Transverse section slightly caudad of the plane of figure 8 and including dorsally the eminentia cerebellaris ventralis (*em. cb. v.*) and ventrally a typical secondary trigeminal neurone (*em. V.*). The section is oblique with the right side much farther caudad than the left and the ventral side somewhat farther caudad than the dorsal. Golgi method. $\times 100$.

Fig. 23 Transverse section at the level of the V roots, illustrating three neurones of the eminentia trigemini (*em. V.*). From the same specimen as figure 22, one section farther caudad. Golgi method. $\times 100$.

Fig. 24 Transverse section at the level of the V roots, illustrating a typical neurone of the eminentia trigemini (*em. V.*). Golgi method. $\times 100$.



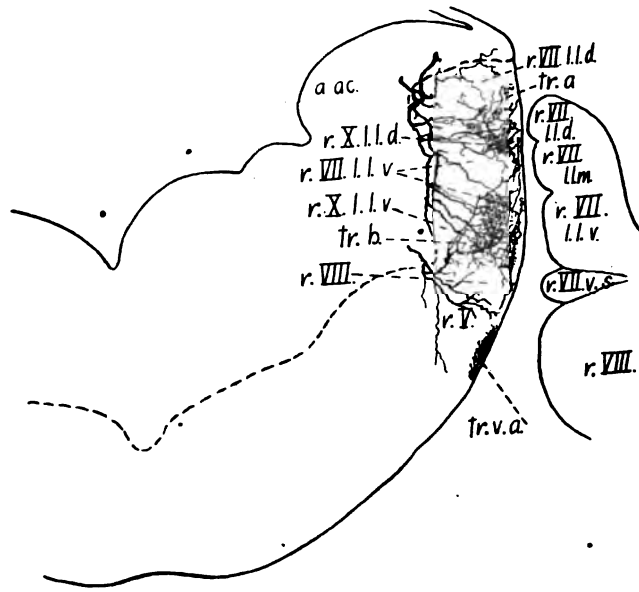


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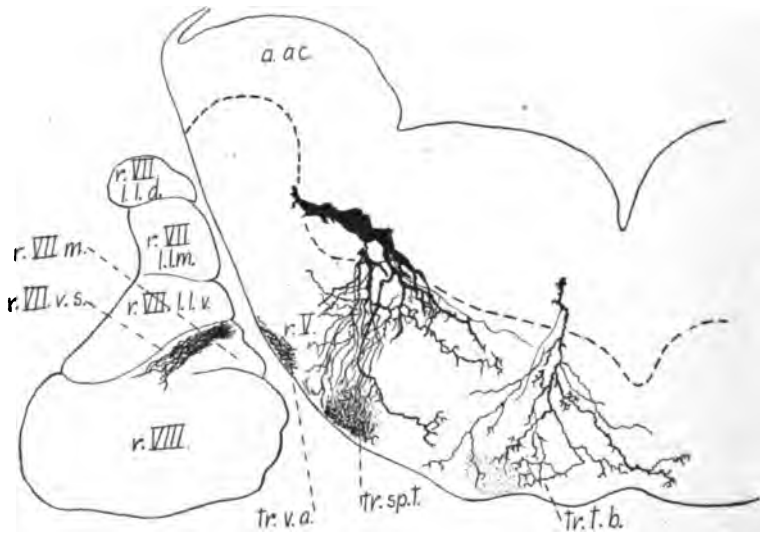
Fig. 25 Transverse section at the level of the V roots, illustrating the relations of neurones of the eminentia trigemini (*em.V.*) to both the V roots and the tractus spino-bulbaris (*tr.sp.b.*). Golgi method. $\times 100$.

Fig. 26 Transverse section immediately caudad of the V roots, showing incomplete impregnations of one small neurone of the sensory V nucleus (*em.V.*) and a tangential neurone of the marginal zone of the gray substance whose dendrites are directed toward the area acustico-lateralis, the sensory V root and the tegmentum. On the opposite side of this section and in adjacent sections larger neurones of the eminentia trigemini send dendrites into the sensory V root and the entire tegmentum, particularly the regions of the tractus spino-bulbaris and the bulbar lemniscus. Golgi method. $\times 100$.

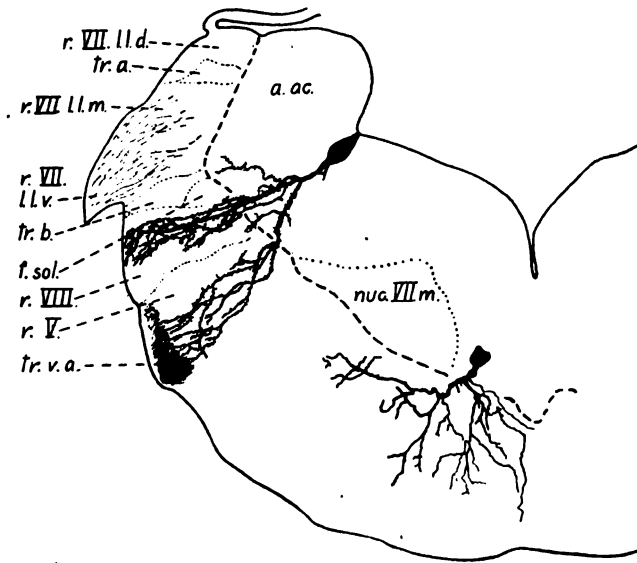
Fig. 27 Transverse section immediately in front of the VII roots. Golgi method. $\times 100$. One neurone of the area acustico-lateralis is partially impregnated. Its axon arises from the ventral dendrite and extends toward the ventral commissure. The dendrites reach the VIII and V roots and the secondary visceral tract (*tr.v.a.*). Two small neurones of the tegmentum (formatio reticularis type) are also impregnated, their axones being directed into the ventral commissure and their dendrites into the tegmentum and secondary visceral tract. These small neurones probably discharge into the motor VII nucleus of the same and the opposite side. The big ventral neurone lies within the area of the motor VII nucleus, but since its axon is not impregnated it is impossible to determine whether it belongs to this nucleus or to the motor tegmentum. Most of its dendrites pass caudad out of the plane of the section. The adjacent section shows them spreading ventrally and laterally throughout the entire tegmental area. Farther caudad in this series some dendrites of a similar neurone reach laterally as far as the spinal V root.



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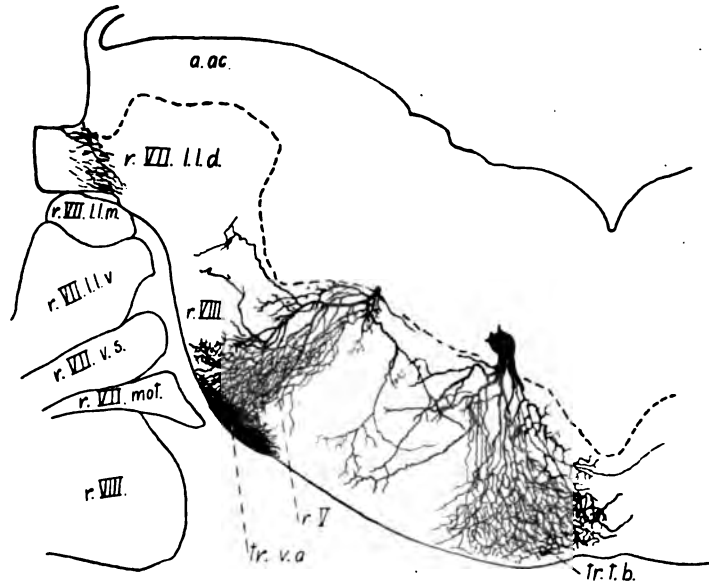


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Fig. 28 Transverse section immediately in front of the VII roots, illustrating a partial impregnation of three neurones of the area acustico-lateralis. Each of the two dorsal neurones is related with the lateralis roots from both the VII and X nerves; the ventral neurone reaches also the VIII and V roots. The section is oblique, the right and dorsal sides being farther caudad. Golgi method. $\times 100$.

Fig. 29 Transverse section immediately in front of the lateralis VII roots. The preparation shows one large tangential neurone of the marginal zone of the gray substance, whose ventral dendrites reach the tegmentum and spinal V root and whose dorsal dendrites (not shown in the preparation) probably spread throughout the area acustico-lateralis. The dendrites of two other neurones are impregnated, one of the substantia gelatinosa Rolandi and one of the motor tegmentum. The latter arborizes chiefly in the tractus tecto-bulbaris of the same and the opposite side. Golgi method. $\times 100$.

Fig. 30 Transverse section at the level of the lateralis VII roots. One neurone of the motor tegmentum is impregnated and farther laterally a typical ependymal element. Golgi method. $\times 100$.

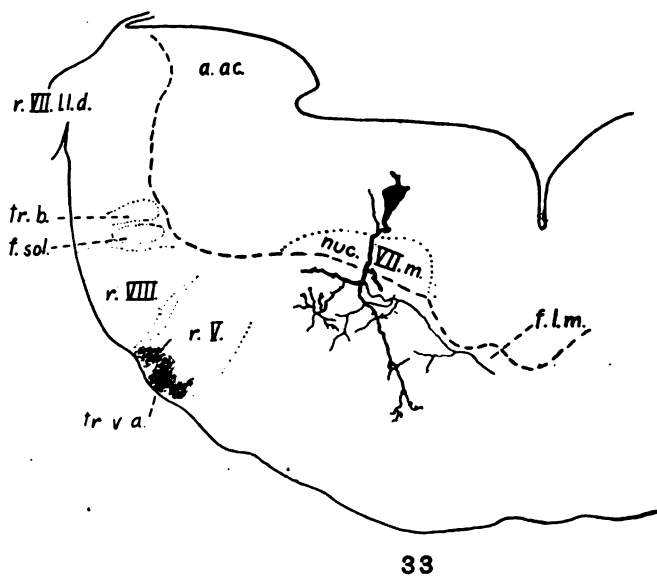
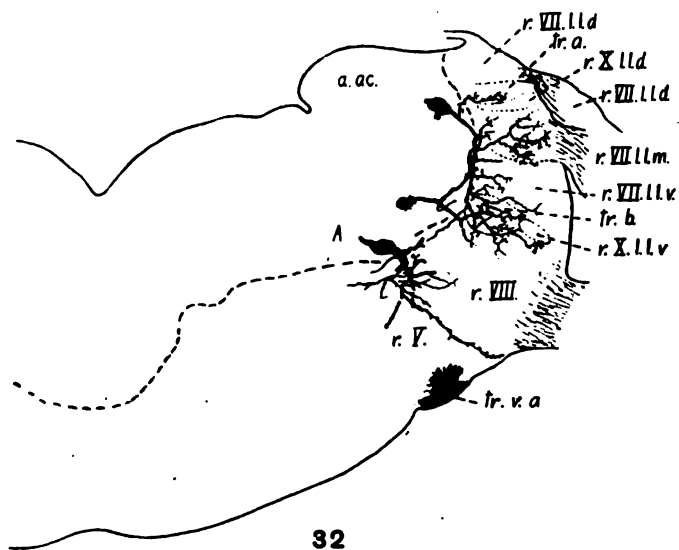


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Fig. 31 Transverse section at the rostral border of the lateralis VII roots, the section being oblique with the left side farther rostrad. Golgi method. $\times 100$. Two neurones are impregnated, the axon in each case being directed toward the ventral commissure. The more lateral neurone is typical for the substantia gelatinosa Rolandi, the chief dendrites arborizing in the spinal V root. Other dendrites, however, reach the VIII root, the secondary visceral tract and the tegmentum. The more ventral neurone is typical for the motor tegmentum, the chief dendrites arborizing in the tractus tecto-bulbaris.

Fig. 32 Transverse section at the level of the VII and VIII roots. Two neurones of the area acustico-lateralis are seen sending their dendrites into particular fascicles of the stratum album. Neurone A, related chiefly with the VIII root, is sketched in from the adjacent section rostrad. Golgi method. $\times 100$.

Fig. 33 Transverse section at the rostral border of the lateralis VII roots, illustrating a single neurone of the tegmentum (formatio reticularis type). Its dendrites reach the lateral part of the tegmentum and still larger ones (cut off in the preparation) spread out in the marginal zone of the gray substance among the arcuate fibers. The axon is directed into the ventral commissure and gives off numerous collaterals close to the cell body. Golgi method. $\times 100$.



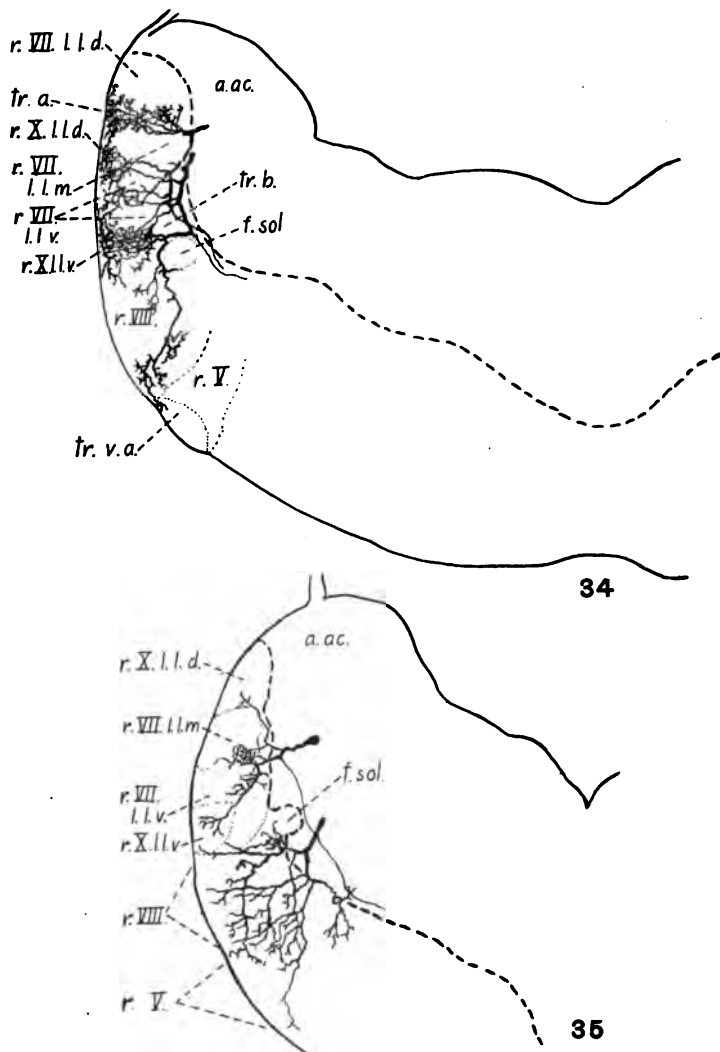


Fig. 34 Transverse section immediately caudad of the VIII root, very oblique, the dorsal side being farther rostrad. Two neurones are partially impregnated whose dendrites spread widely throughout the area acustico-lateralis but are related with particular tracts in a very specific way. Golgi method. $\times 100$.

Fig. 35 Transverse section a short distance rostrad of the IX roots, illustrating two neurones of the area acustico-lateralis. One is related with both dorsal and ventral lateral X roots and with two of the lateralis VII roots; the other chiefly with the VIII and V roots. Golgi method. $\times 100$.

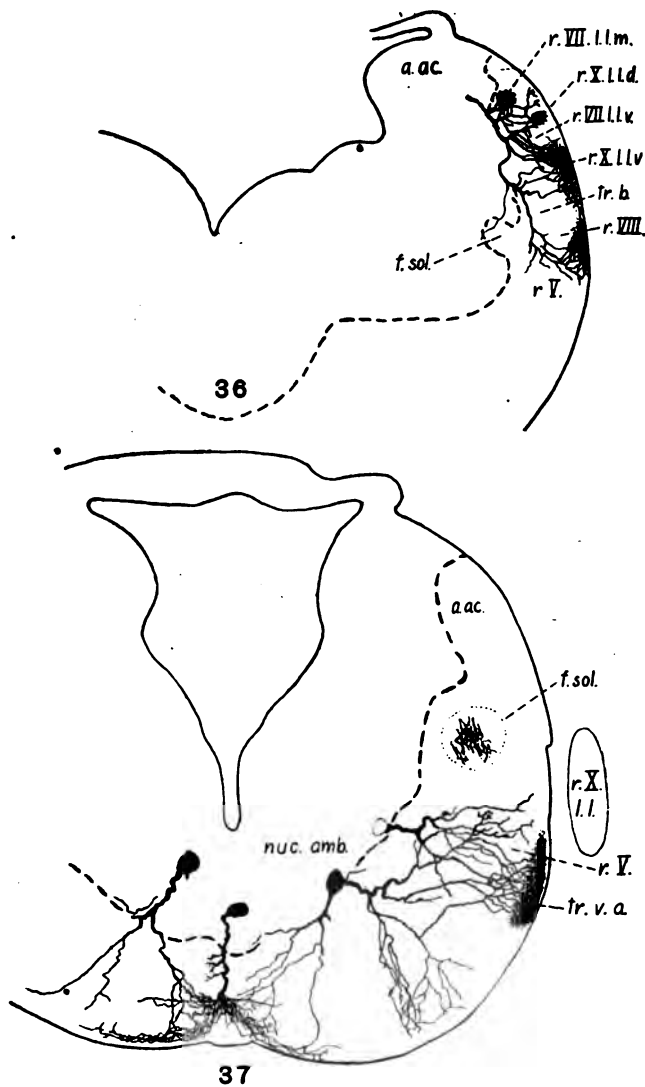
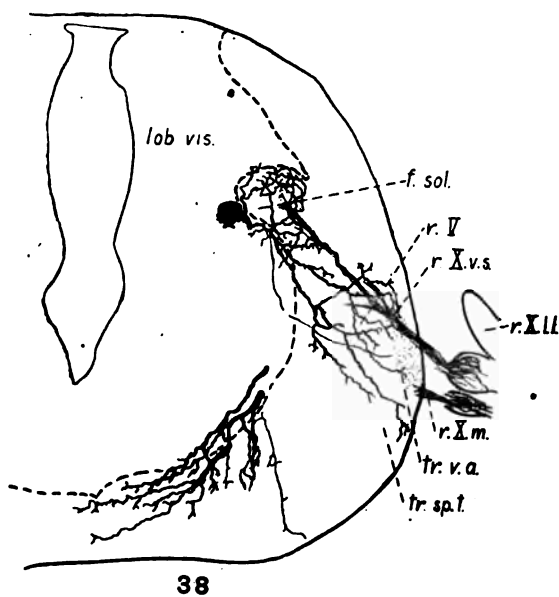
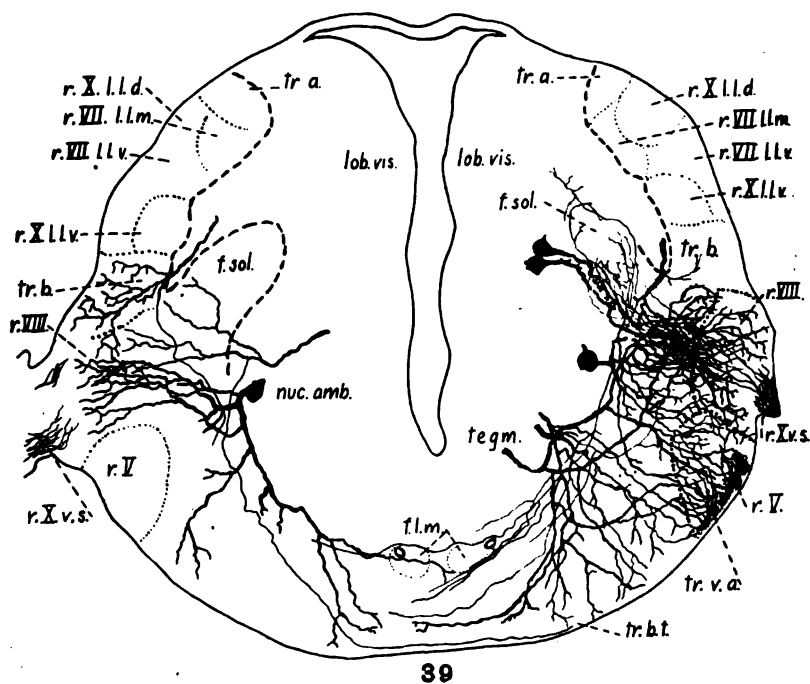


Fig. 36 Transverse section a short distance rostral of the IX roots, illustrating a single neurone of the area acustico-lateralis whose dendrites reach specific regions of the stratum album containing special fascicles of root fibers. Golgi method. $\times 100$.

Fig. 37 Transverse section between the IX and X roots, illustrating ventrally two neurones of the motor tegmentum and farther laterally two which probably belong to the nucleus ambiguus. The latter appear to effect their chief connections with the secondary visceral tract (*tr.v.a.*), and also with the spinal V root and the tegmentum farther ventrally. Golgi method. $\times 100$.



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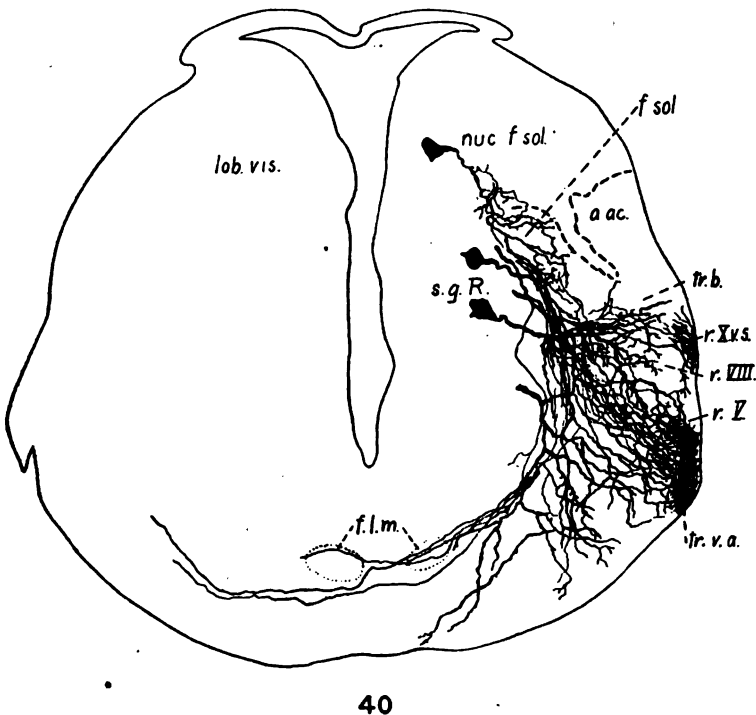
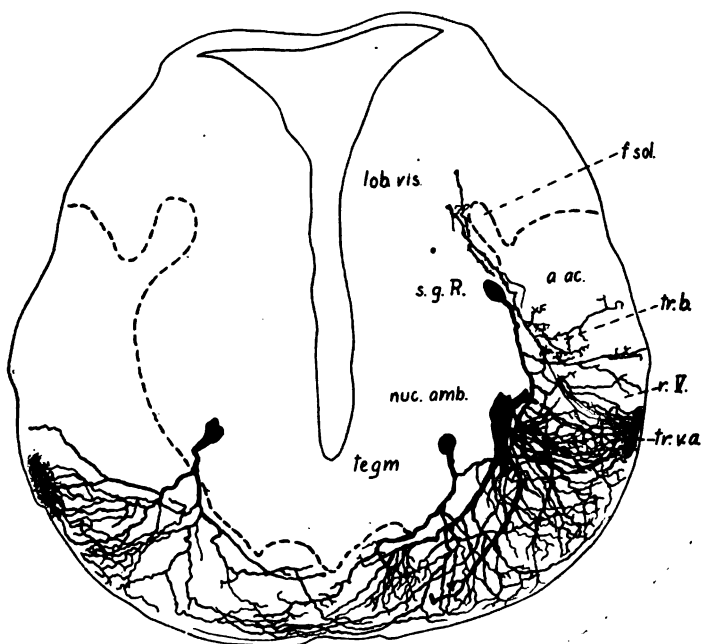


Fig. 38 Transverse section at the level of the vagus roots, illustrating a neurone whose dendrites are related both to the fasciculus solitarius and to the spinal V root. The axon is directed into the secondary visceral tract. Other sections in this region show similar neurones whose axones divide, one branch entering the secondary visceral tract of the same side and the other entering the ventral commissure; cf. also figure 40. This preparation also shows dendrites of two neurones of the motor tegmentum. Golgi method. $\times 100$.

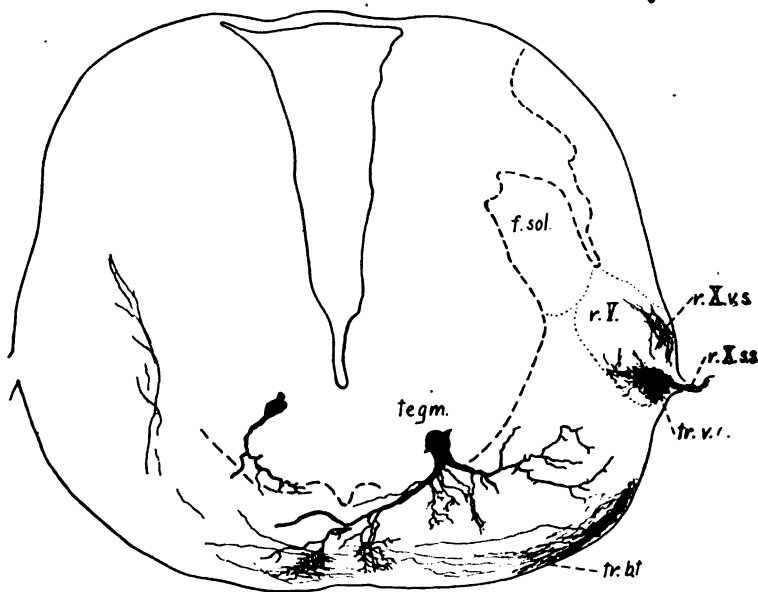
Fig. 39 Transverse section at the level of the vagus roots, illustrating (on the right) the mode of termination of the visceral sensory vagus roots (*r.X.v.s.*) in the fasciculus solitarius and various types of neurones of the tegmentum. Golgi method. $\times 100$.

Figures 39, 40 and 41 are consecutive sections from the same specimen.

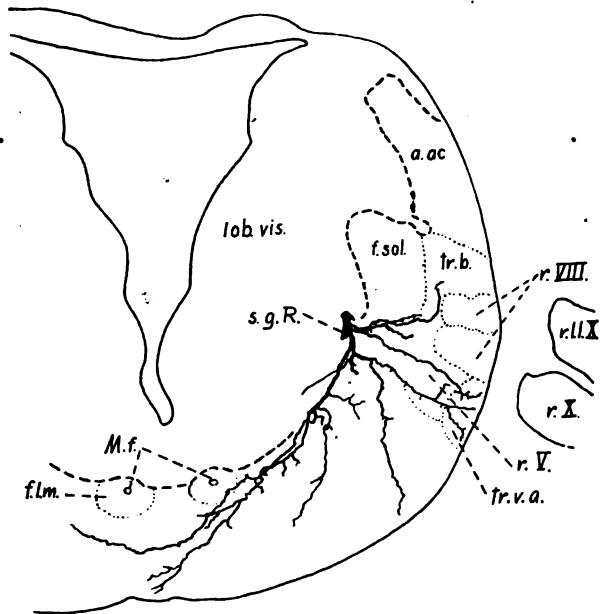
Fig. 40 Transverse section at the level of the vagus roots, illustrating dorsally a typical neurone related to the fasciculus solitarius and immediately ventrally of this fasciculus several neurones of the substantia gelatinosa Rolandi (*s.g.R.*) whose dendrites reach the VIII root, the spinal V root, the secondary visceral tract and the underlying tegmentum. The axones of the latter neurones enter the ventral commissure; those from the nucleus of the fasciculus solitarius enter the secondary visceral tract (*tr.v.a.*) and probably also send branches into the ventral commissure. Golgi method. $\times 100$.



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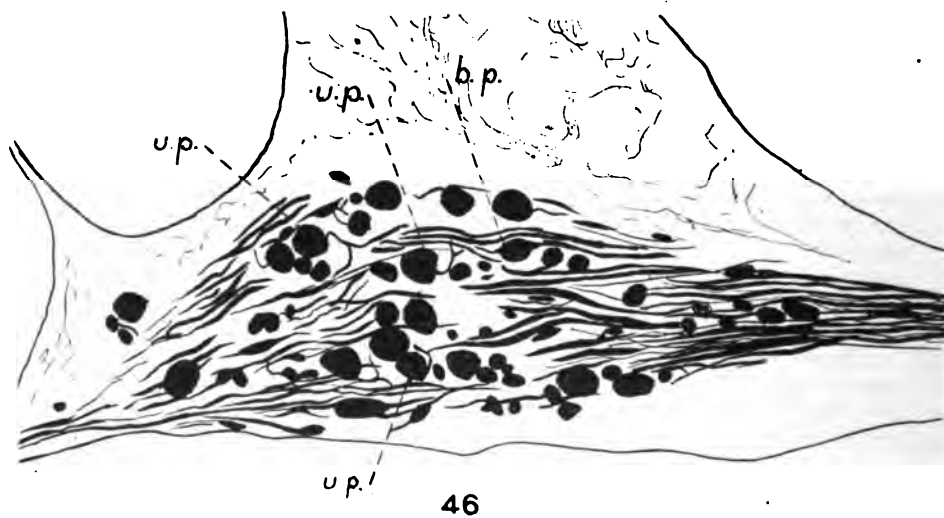
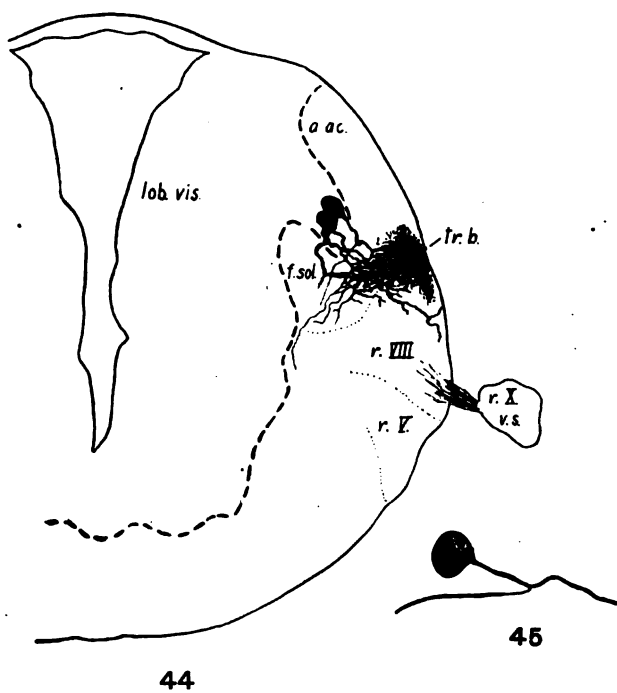


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Fig. 41 Transverse section at the level of the vagus roots, showing dendrites of two neurones of the nucleus of the fasciculus solitarius, a neurone of the substantia gelatinosa Rolandi immediately ventrally of these, farther ventrally a neurone of the nucleus ambiguus, and still more ventrally two neurones of the motor tegmentum. Golgi method. $\times 100$.

Fig. 42 Transverse section at the level of the vagus roots, illustrating two neurones of the motor tegmentum. Golgi method. $\times 100$. Sections of this series farther caudad show dendrites of similar neurones extending dorsalward as far as the lateralis X roots, giving off branches to all regions ventrally of this level except the fasciculus solitarius. These are similar to the typical neurones of the motor tegmentum of the spinal cord. The fibers which are here seen entering the tractus bulbo-tectalis are shown by sections farther caudad to arise from the region immediately ventrally of the spinal V root.

Fig. 43 Transverse section at the level of the vagus roots, illustrating a single neurone of the substantia gelatinosa Rolandi whose dendrites also reach the entire tegmental region. Golgi method. $\times 100$.



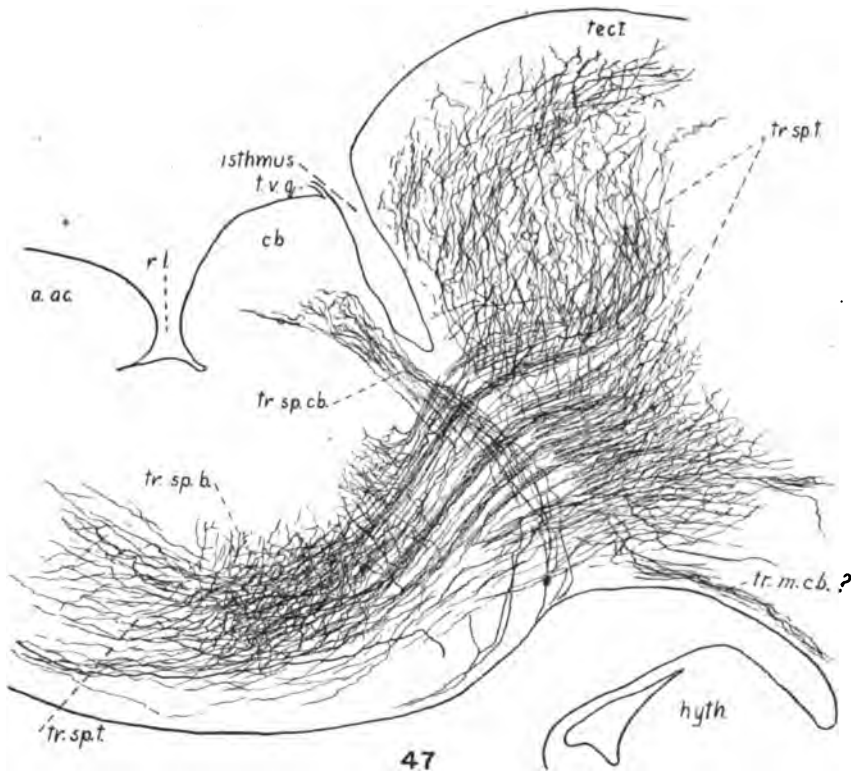


Fig. 44 Transverse section at the level of the vagus roots, illustrating two neurones related with correlation tract *b*. Golgi method. $\times 100$. Other sections show similar neurones which send dendrites into tract *b* and also into the area acustico-lateralis farther dorsally.

Fig. 45 A neurone of the ganglion semilunare (Gasseri) of larval *Amblystoma*, from the same specimen as figure 56. The central process is directed to the left, the peripheral process to the right. Method of Ramón y Cajal. $\times 225$.

Fig. 46 Horizontal section through the left anterior lateralis VII ganglion of a 38 mm. larva, illustrating unipolar and bipolar neurones of large and small size. Most of the small oval nuclei of the neurilemma are omitted from the drawing. The proximal end of the ganglion is at the right. Method of Ramón y Cajal. $\times 200$.

Fig. 47 Parasagittal section through the isthmus region illustrating the way in which fibers of the spinal lemniscus complex (*tr.sp.t.*) give off collaterals to the tegmentum of the oblongata (*tr.sp.b.*), to the cerebellum (*tr.sp.cb.*), and to the tectum (*tr.sp.t.*). Golgi method. $\times 100$.

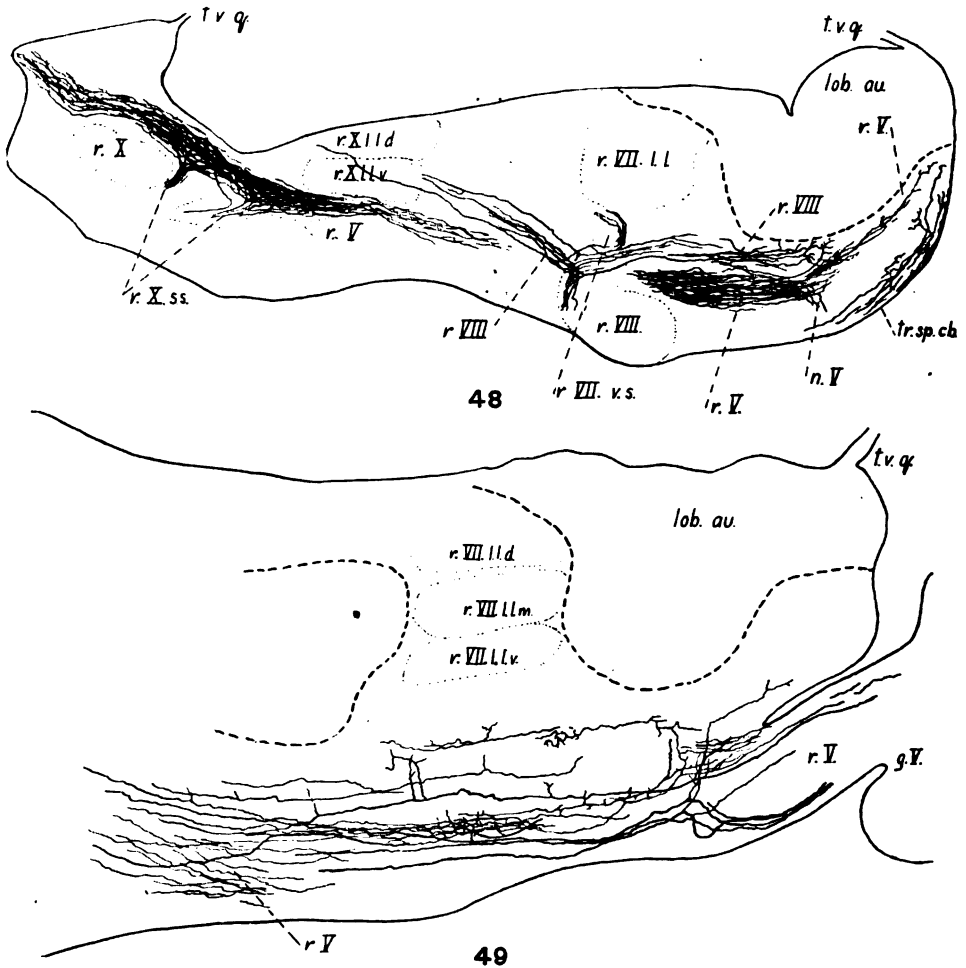


Fig. 48 Parasagittal section through the medulla oblongata, illustrating the arrangement of fibers of the V, VIII and X roots. Golgi method. $\times 67$. The heavy broken line marks the boundary of the stratum griseum of the auricular lobe, the fine dotted lines the positions of the cranial nerve roots. At *n.V* is indicated the point of entrance of a few fibers from the sensory V root, which immediately divide into descending and ascending branches (*r.V.*), the latter terminating in free arborizations at the rostral end of the auricular lobe. Root fibers of the VIII nerve are seen to bifurcate into ascending and descending branches (*r.VIII.*), and farther caudad root fibers of two cutaneous roots of the vagus (*r.X.s.s.*) join the spinal V root (*r.V.*).

Fig. 49 Details of the terminals of the sensory V root fibers, as seen in parasagittal section. Golgi method. $\times 100$.

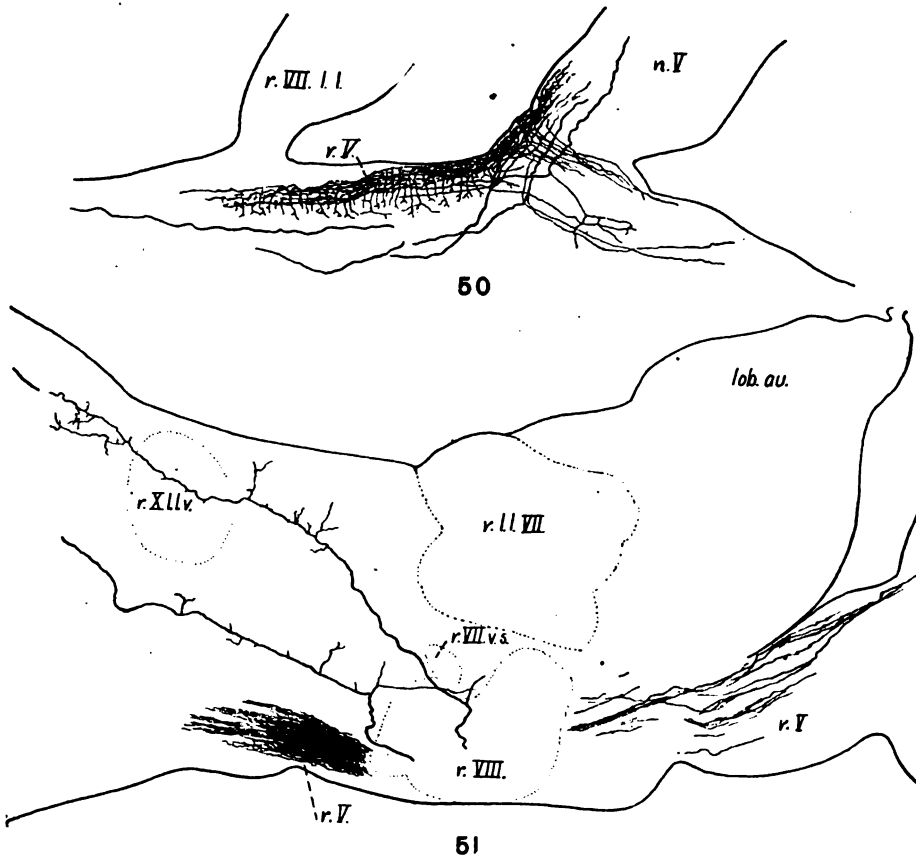


Fig. 50 Detail of the relation of fibers of the sensory root of the trigeminus as seen in horizontal section of the left side. Golgi method. $\times 100$. The figure is a composite, the outline and the three coarse fibers being drawn from one preparation and the fascicle of fine fibers sketched in from the adjacent section ventrally. The rostral end of the oblongata is at the right and the root fibers are seen to divide into slender ascending branches and coarser descending branches which form the spinal V root (*r.V.*)

Fig. 51 Parasagittal section through the oblongata, illustrating two root fibers of the VIII nerve. Golgi method. $\times 100$. The section is very close to the lateral surface of the oblongata and is obliquely inclined. Each of the two root fibers (*r.VIII.*) is seen to divide into ascending and descending branches and the details of the terminals of the descending branches are shown.

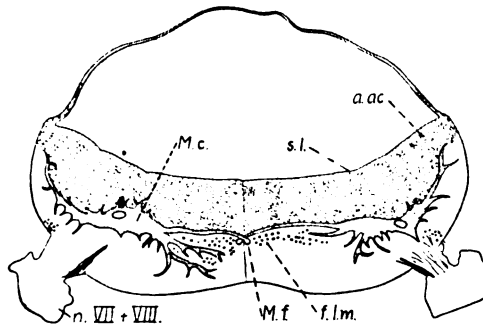
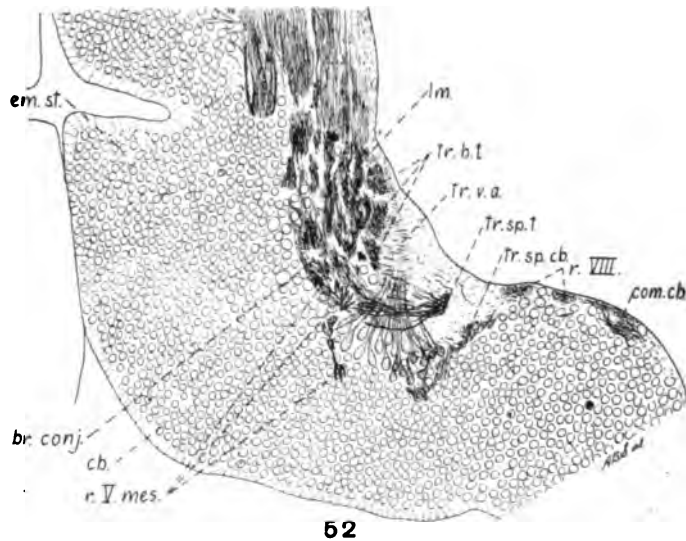


Fig. 52 Part of a horizontal section through the brain of a 54 mm. larva of *Amblystoma* prepared by the method of Ramón y Cajal. $\times 100$. The section is somewhat oblique, the right side being farther dorsal. For plane of section, see figure 6. The section illustrates the relations of a group of neurones in the base of the cerebellum to the neuropil which receives the ascending secondary visceral tract (*tr. v. a.*) and which probably corresponds with the "Rindenknotten" of Mayser in teleosts. For the relations of the termini of VIII root fibers (*r. VIII.*) in this figure, cf. figure 6.

Fig. 53 Sketch of a cross section through the medulla oblongata at the level of the VII and VIII roots of a 12 mm. larva of *Amblystoma tigrinum*. $\times 74$. In the sketch the complete outlines of Mauthner's cells and their chief processes have been reconstructed by the superposition of camera lucida outlines of the seven adjacent sections within which parts of the cell are found, the sections being 5μ thick.

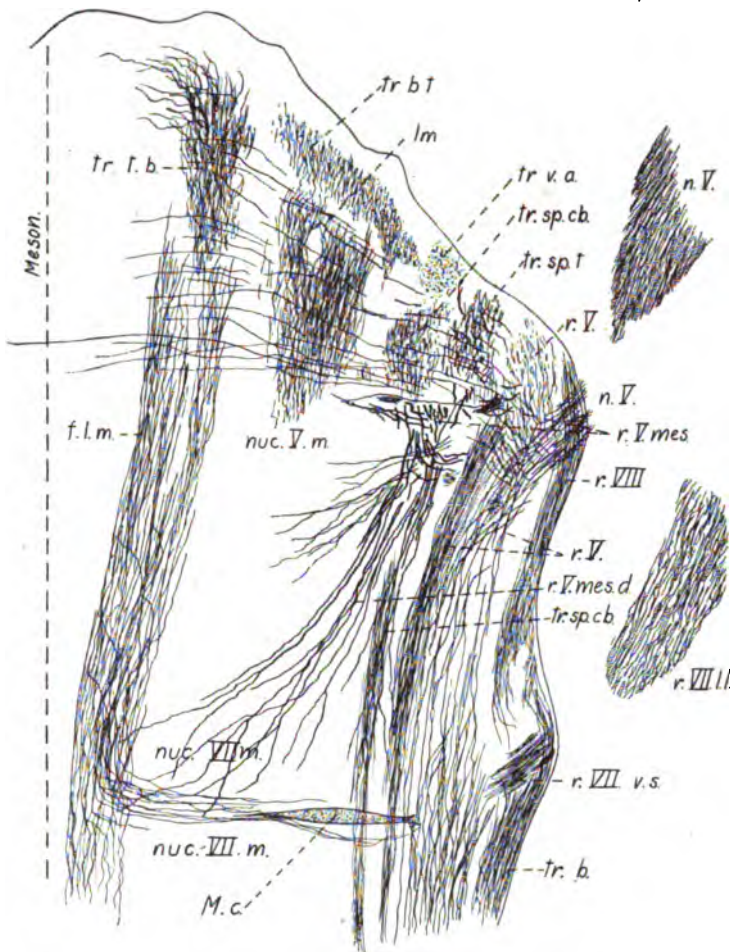


Fig. 54 Part of a horizontal section (slightly inclined with the right side farther dorsal) through the medulla oblongata of larval *Amblystoma tigrinum* 54 mm. long; silver impregnation method of Ramón y Cajal. $\times 100$. The fibers of the mesencephalic V root are seen near the superficial origin of the V roots, where they separate from the spinal V root to turn inward and forward across the rostral aspect of the motor V nucleus. The positions of the motor V and motor VII nuclei are indicated on the drawing, though the cells of these nuclei lie immediately dorsally of the plane here figured. The entire region between the V and VII roots in this preparation is filled with arcuate fibers, which have not been sketched in the figure. The fibers of the mesencephalic V root are coarser than any others of this region and can be readily distinguished. They are seen to give off equally coarse collateral branches which terminate in the motor VII nucleus. In some of our sections much finer branches of these fibers are seen to terminate in the motor V nucleus, but these are not visible in this preparation.

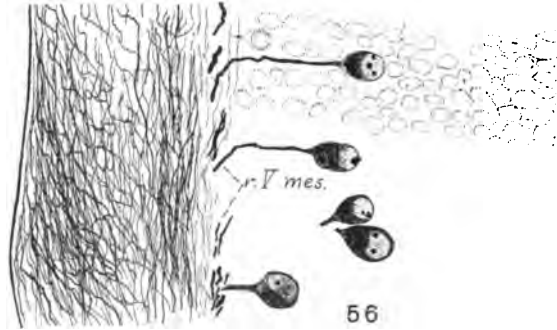
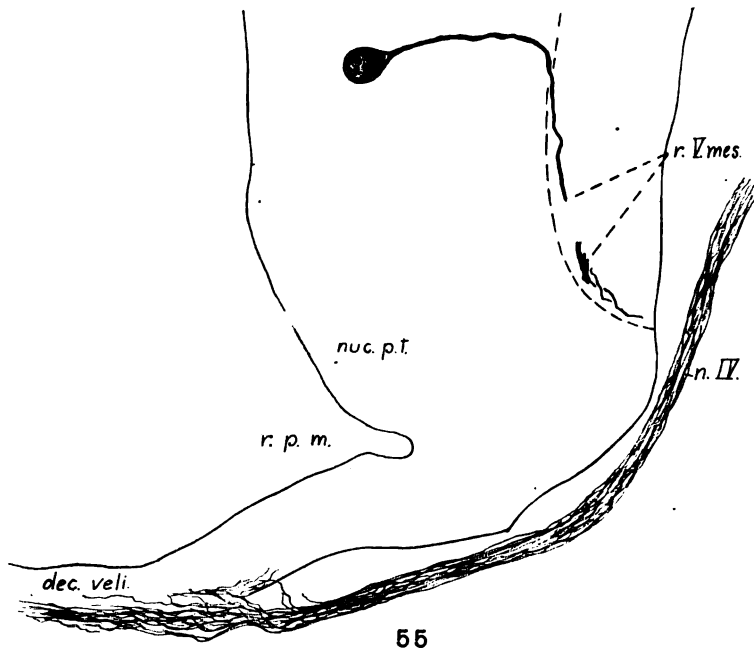


Fig. 55 Neurone of the nucleus magnocellularis tecti, giving rise to a fiber of the radix mesencephalica trigemini: From the right side of a horizontal section through the tectum mesencephali of an *Amblystoma* larva of 40 mm. prepared by the method of Ramón y Cajal. $\times 225$.

Fig. 56 Five neurones of the nucleus magnocellularis tecti, giving rise to fibers of the radix mesencephalica trigemini. Drawn from the left side of a horizontal section through the tectum of an *Amblystoma* larva 45 mm. long prepared by the method of Ramón y Cajal. The ependymal surface is at the left, the pial surface at the right, and the caudal end of the section is below. For purposes of orientation the nuclei of the other neurones of the tectum are sketched in dotted outlines in part of the figure. $\times 225$.

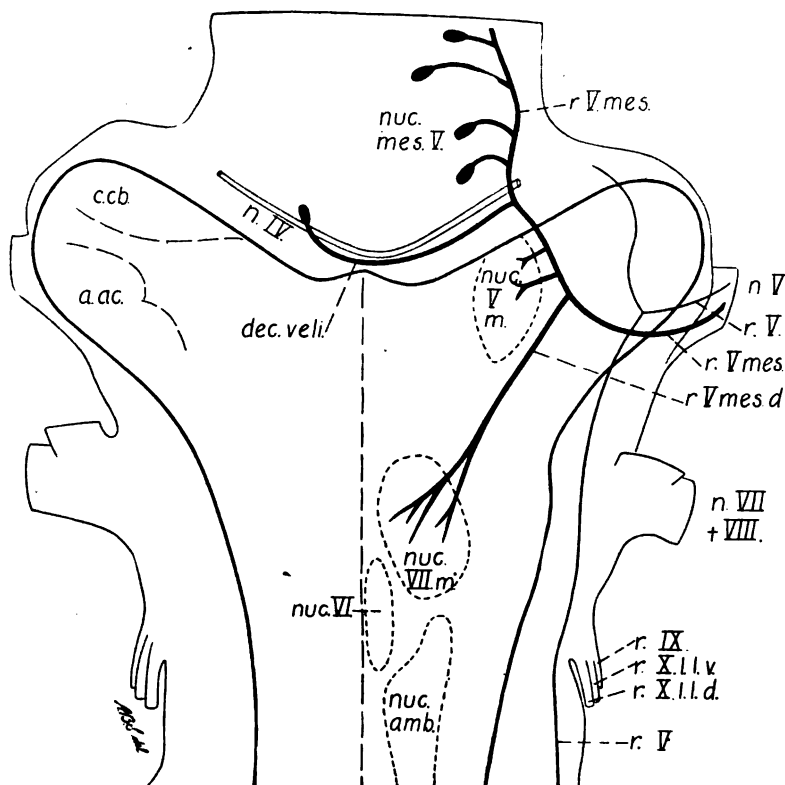


Fig. 57 Diagram of the connections of the mesencephalic root of the trigeminus in larval *Amblystoma*. The outline of the sketch is taken from the dorsal view of the model of a 38 mm. larva shown in figure 1. The individual fibers of the root arise from cells of the nucleus magnocellularis tecti (*nuc. mes. V.*), those from the caudal part of the tectum in part decussating in the commissura veli in company with the IV nerve and the cerebellar commissure. The root then descends in several strands through the body of the cerebellum and along the rostral and lateral border of the motor V nucleus. Laterally of this nucleus many of the fibers of this root divide into two branches each of which is as thick as the parent stem. One of these branches is directed backward and inward to end by free arborizations in the motor VII nucleus; the other enters the peripheral root of the V nerve. Other finer collateral branches of the root fibers enter the motor V nucleus.

THE PRESENCE OF MEDULLATED NERVE FIBERS
PASSING FROM THE SPINAL GANGLION TO
THE VENTRAL ROOT IN THE FROG,
RANA PIFIENS¹

ELIZABETH HOPKINS DUNN

*From the Hull Laboratory of Anatomy of the University of Chicago, and The Nelson
Morris Institute of Medical Research*

ONE FIGURE

It seems advisable at the present time to make a brief report of the presence in the nervous system of *Rana pipiens* of a group of medullated nerve fibers appearing in the ventral root which apparently arises in the spinal ganglion.

Recognition of this group of nerve fibers occurred in the course of a study in the frog (Dunn '09) of the distribution of the peripheral medullated nerve fibers after unilateral section of the ventral roots of the VIIIth, IXth, and Xth spinal nerves, Gaupp's nomenclature. It was found that after degeneration should have occurred in all the ventral root nerve fibers which were out-growths of ventral column neurones in the spinal cord, some intact medullated nerve fibers were found in each root. The medullated nerve fibers in two of the ventral roots were scattered, but fortunately in the ventral root of the Xth spinal nerve some of the fibers were grouped in two bundles and could be traced to the Xth spinal ganglion.

In this frog the ventral roots had been torn loose at their points of emergence from the spinal cord and were left free in the spinal canal. Because of their preservation by this method of operating the ventral roots could be examined for their full extent. The operation was performed eight months before the frog was

¹ Reported at the meeting of the American Association of Anatomists in the winter of 1910.

killed. Presumably complete degeneration was accomplished. The dorsal roots and the spinal ganglia were found intact.

The bundles of nerve fibers now under consideration extended from the central end of the ventral root into the spinal ganglion. Their position at the central end of the ventral root was near the center of the cross section of the root. At the point where the ventral root became applied against the dorsal root with a connective tissue septum intervening, the fused bundle turned at almost a right angle and plunged through the substance of the dorsal root and into the spinal ganglion. As a bundle it could be followed dorsally into the ganglion and within the ganglion laterally into a region where the medullated nerve fibers

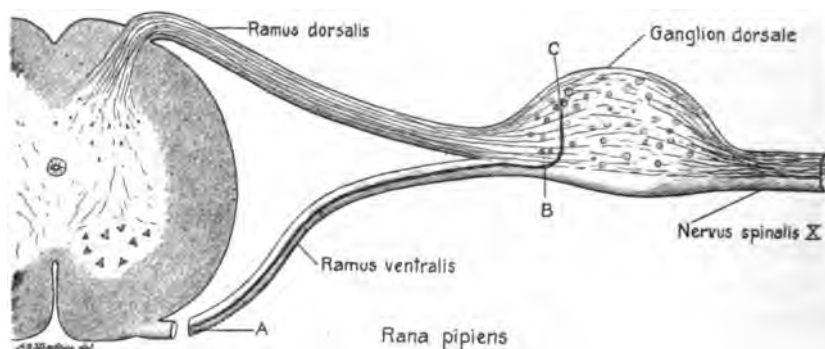


FIGURE 1

were few and the perikarya numerous. The derivation of these fibers from adjacent perikarya seems a fair assumption. The course of these fibers and their relation to the ganglion is shown diagrammatically in figure 1. While following the fibers serially from A to C, their course from B to C was found to be accomplished within a dozen sections of ten micra thickness.

In all, some twenty medullated nerve fibers of varying sizes could be traced to the spinal ganglion of the Xth nerve. On direct measurement, the fibers fell into two groups according to their diameter, those with a diameter of more than ten micra, and those with a diameter of less than five and five-tenths micra. Of the first group, those with a diameter of more than ten micra,

the largest had a diameter of thirteen micra, the remainder had a diameter intermediate between thirteen and ten micra. Of the second group, the smallest fibers had a diameter of four micra. For comparison with these measurements the size of the central fibers of the dorsal root was determined. The largest fibers in the dorsal root of this Xth nerve had a mean diameter of eighteen micra and the smallest fibers a mean diameter of less than two micra. If the fibers under discussion are central fibers from the dorsal root ganglion it follows that they are neither the largest nor the smallest of the dorsal ganglion fibers, but are intermediate in size.

The preservation of these undegenerated medullated nerve fibers after complete and prolonged separation of the ventral root from the spinal cord, and their relations to other structures in the ventral root seem sufficient evidence of the peripheral location of their perikarya. If the validity of this evidence is admitted the question of the location of the perikarya at once arises. Peripheral nerve cells are found in the spinal ganglia and in the sympathetic ganglia. I am inclined to interpret the origin of this group of nerve fibers as from the spinal ganglia because of their relation to the structures within the ganglion. If these fibers in the ventral root originate in sympathetic ganglia, their central course must be by way of the peripheral portion of the dorsal root, as careful search revealed no medullated nerve fibers, large or small, entering the ventral root from the peripheral nerve. The size, also, is greater than the size attributed to the postganglionic medullated nerve fibers of Langley. Reference has already been made to the close association of these fibers with the perikarya at the periphery of the ganglion.

For an interpretation of the functional significance of such nerve fibers as have been noted in this frog it is necessary to pass in review the findings of others who have noted the presence of atypical fibers in the ventral or dorsal roots.

Sherrington ('94) noted the presence of undegenerated medullated nerve fibers in the lumbo-sacral roots of the monkey and the cat. Sherrington interprets these ventral root fibers as having their origin in the peripheral portion of the posterior

(dorsal) root and terms them recurrent fibers. He suggests that they afford the basis of the familiar recurrent sensibility noted by Schiff and Bernard. That is, Sherrington judges the fibers he found to be fibers carrying sensation from the ventral root to the spinal ganglion and thence to the spinal cord. Sherrington traces the fibers to the dorsal root near the exit of the root into the spinal nerve.

Kidd ('11) has assumed the presence in the ventral root of certain afferent nerve fibers which are supposed to have their perikarya within the spinal cord. Kidd's contention is that afferent nerve fibers are present in the ventral root of the spinal cord of man and other vertebrates and that their origin lies within the spinal cord from endoneural ganglion cells located in (1) the smaller cells of Clarke's column, (2) in the solitary cells of the dorsal horn, (3) some of the 'middle cells' of the cord, and possibly also (4) some of the cells of the nucleus cuneatus of the bulb.

Braeunig ('03) noted the existence of early degeneration of certain fibers in the ventral nerve root after section of the dorsal root. Braeunig assumes that the section of the dorsal root may cause degeneration of certain of the motor cells within the spinal cord and of their axis cylinder processes which would be found in the ventral root as efferent fibers.

In the present investigation the medullated nerve fibers were followed in serial section through the mass of dorsal root fibers into the substance of the ganglion and to a part of the ganglion much nearer to the central end of the ganglion than in the case of Sherrington's group of nerve fibers. For this reason I am inclined to interpret such fibers as central, not as peripheral processes of the spinal ganglion neurones. Their location in the ventral root at its central end must also be taken into consideration. If the function of these fibers is the carrying of sensory impressions from the substance of the spinal cord or from the meninges, one would expect to find them near the enveloping membranes of the root.

These fibers cannot be of the type to which Kidd refers. If they were processes of endoneural cells which left the spinal

cord by way of the ventral root they would have degenerated when their central connections were severed.

If fibers of this group were closely connected with fibers in the dorsal root, section of the dorsal root might produce a degeneration such as was noted by Braeunig. This connection might be of two types. The recurrent fibers of Sherrington might be affected by retrograde degeneration if their central processes in the dorsal root were severed. Or, if branching fibers occur in which one process enters the spinal cord by the dorsal root and the second by the ventral root, a similar degeneration might follow section of the dorsal root.

It remains for us to consider the possibility of localizing within the spinal ganglion the neurone bodies with which our medullated nerve fibers are united. We are able to refer to certain experiments in which the same region of the spinal ganglion is affected. Kleist ('04) after section of certain dorsal roots found that in a region on the posterior aspect of the proximal part of the ganglion the degenerative changes in the nerve cells were much more marked than in other parts of the ganglion. The spinal nerves were not cut in Kleist's experiments. The location of this region of extreme degeneration corresponds approximately with that to which I was able to trace the intact ventral root fibers. I am not able, however, to suggest any relation between the presence of intact fibers in the operated ventral root apparently arising in this region and the excessive degeneration noted by Kleist. Such causal relation may exist.

Numerous investigators have noted the presence within the spinal ganglion of certain cells which did not degenerate after section of the spinal nerves. Ranson ('09) in his discussion of alterations in spinal ganglion cells, corroborates the findings of earlier investigators in that he notes the presence of non-reactive nerve cells in the spinal ganglia of the white rat. Ranson describes these cells as of a medium size and with a clear protoplasm with large chromatic granules. These cells are not numerous, constituting a small percentage of the total number of nerve cells in the spinal ganglion and are scattered through the ganglion. Ranson agrees with Cox ('98) and with Warrington and Griffith

('04) in considering that these cells represent the cells of Dogiel's Type II (new types III, IV, VIII and XI) and are relay cells within the ganglion. The scattered condition of these cells is against the identification of them with the perikarya of our fibers. The microscopic characters of the non-reactive cells as described by the various observers do not agree with those of the mass of neurones which are supposed to be connected with the functioning of sensory transmission from the periphery to the spinal cord. I am able therefore to make no suggestions as to the identification of the perikarya under discussion with observed neurones within the spinal ganglia.

Finally, one physiological finding may be cited in connection with the contention that these are central sensory fibers. It has been noted that sensory stimuli have reached the central nervous system after the dorsal roots no longer function. This occurs in cases of disease of the dorsal roots, but its recognized association with the experimental interruption of function is of more importance to this discussion. The existence of central processes of spinal ganglion neurones may furnish the pathway over which such sensory impulses find their entrance to the central nervous system. If this pathway exists Kidd's postulate of the occurrence of afferent ventral root nerve fibers of central location is unnecessary. The presence in the dorsal root of aberrant fibers of efferent function was determined through physiological experiment by Horton-Smith in 1897.

Aberrant medullated nerve fibers have been discovered in many parts of the central nervous system and seem to be as frequent in occurrence as might be postulated from our knowledge of the early stages of development of the neurone. Since axis cylinder processes are outgrowths of neurones of tentacle like nature which seek for and are perpetuated by permanent functional connections, it may well be that some of these tentacles in the case of spinal ganglion neurones push their way among the embryonic connective tissue structures, and on reaching the ventral root outgrowth, follow the matrix thus furnished and arrive at the spinal cord. Such axonic processes as find

functional relations within the spinal cord persist and continue as ventral root fibers.

The aberrancy or anomalous distribution of peripheral axones warns us against the making of functional interpretations based altogether on the location of neurones or their processes. I have had occasion to report the existence of dividing sensory nerve fibers in *Rana pipiens*, one branch of which passed to so-called visceral structures and the other to so-called somatic structures. Such distributions raise the question of the value of connoting significance to the distribution of nerve fibers save as a convenient form of generalization. Ventral root fibers are not all motor. Sensory fibers are not all visceral or somatic.

Such anatomical findings as are presented in this paper may be of value in checking up unusual physiological or pathological observations. Their significance is not vitiated by the paucity of fibers in comparison with the great masses of fibers in the dorsal root. Complexity of distribution is a factor which must be considered in the interpretation of the value of a conducting pathway.

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GANGLION CELLS OF THE NERVUS TERMINALIS IN THE DOGFISH (MUSTELUS CANIS)

PAUL S. McKIBBEN

From the Anatomical Laboratory of the University of Chicago

SIX FIGURES

Within the last few years the nervus terminalis has furnished the material for many observations. This nerve has now been studied in fishes, where it was first described, in amphibians, reptiles and mammals. In a few of these studies, cells have been observed associated with the nerve. In some cases these cells are grouped into well-marked ganglia.

In Locy's description of the nerve in twenty-seven species of selachians (Locy '05) a well-marked ganglion was found situated on the nerve usually near the olfactory bulb. It was also noted by Locy that there might be two ganglia on a single nerve with minute accessory clusters of cells. "In general structure," Locy ('05) has stated, "one of these ganglia resembles a spinal ganglion." Further, "In the ganglia so far studied, there is a preponderance of bipolar nerve cells, but, in a few cases, other ganglion cells have been observed with angular outlines and three (or more) processes, suggesting the presence of a limited number of multipolar cells." In this study Locy dealt with sections of these ganglia, from *Squalus* and *Alopias*, stained by the usual histological methods.

Through the kindness of the Commissioner of Fisheries and the Director of the Biological Laboratory at the Wood's Hole Station of the United States Bureau of Fisheries, opportunity has been afforded the author for studying the nervus terminalis of the live dogfish. The purpose of the present contribution is to report the appearance of the cells of the ganglion of the nervus terminalis after treatment with methylene blue.

The material used consisted of adult specimens of the smooth dogfish (*Mustelus canis*) and spiny dogfish 'pups' (*Squalus acanthias*). Adult specimens of *Mustelus canis* have been used as the basis for all the figures and descriptions which follow.

The fish were taken from the water, strapped in a specially constructed trough and clamped at the root of the tail. The tail was then cut off about two centimeters caudal to the cloacal aperture and a solution of methylene blue in salt solution injected with a pressure bottle into the caudal artery. A 0.075 per cent solution of methylene blue (Grübler's "reét., nach Ehrlich") in 2 per cent salt solution was found most useful. After a successful injection of the nasal region the mucous surface of the nostrils was found to be colored quite blue. The head of the fish was now cut off, the cranium opened and the nervus terminalis dissected off with the aid of a stereo-binocular microscope. The nerves were fixed in cold ammonium molybdate, dehydrated rapidly and mounted in balsam or embedded in paraffine. For further details concerning this method see Wilson ('10) and McKibben ('13). About twenty fish were thus injected and the study of thirty whole mounts of separate nerves has furnished the basis for the observations which follow.

For the examination of the whole mounts, the Zeiss binocular stereoscopic ocular used with high power apochromatic objectives was found exceedingly useful. With this apparatus it was found that the continuity and arrangement of the processes of the ganglion cells could be studied much more easily and accurately than with a simple ocular.

The arrangement of the ganglion cells along the nerve varies with each individual and on the two sides of the same head. In three cases nerves were studied which showed a single ganglion. In the others, although usually one group was present which was larger than the rest, the cells were arranged in several ganglia. The main ganglion, as Locy has indicated, is situated usually near the olfactory bulb. Three, four, five and six well-marked ganglia have been observed on a single nerve and in one case ten relatively isolated groups of cells have been encountered.

These may occur at any point along the nerve from the place where it leaves the brain to its entrance into the nasal sac.

In figure 1 appears a drawing which shows the general relations of the cells in the main ganglion of the nervus terminalis. The other figures illustrate single cells. A glance at the figures will indicate that multipolar cells are quite predominant. In a study of the thirty nerves only a few cells have been encountered which might be bipolar in structure and in these the bipolar condition can not be established with certainty. That the selective action of the methylene blue has picked out only the multipolar cells and has left unstained the bipolar cells is not impossible but not at all probable.

CONCLUSION

In the smooth dogfish (*Mustelus canis*) the ganglia of the nervus terminalis, when stained with methylene blue (intra-vitam), are seen to contain multipolar nerve cells. Few, if any, bipolar cells have been demonstrated.

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PLATES 1 AND 2

EXPLANATION OF FIGURES

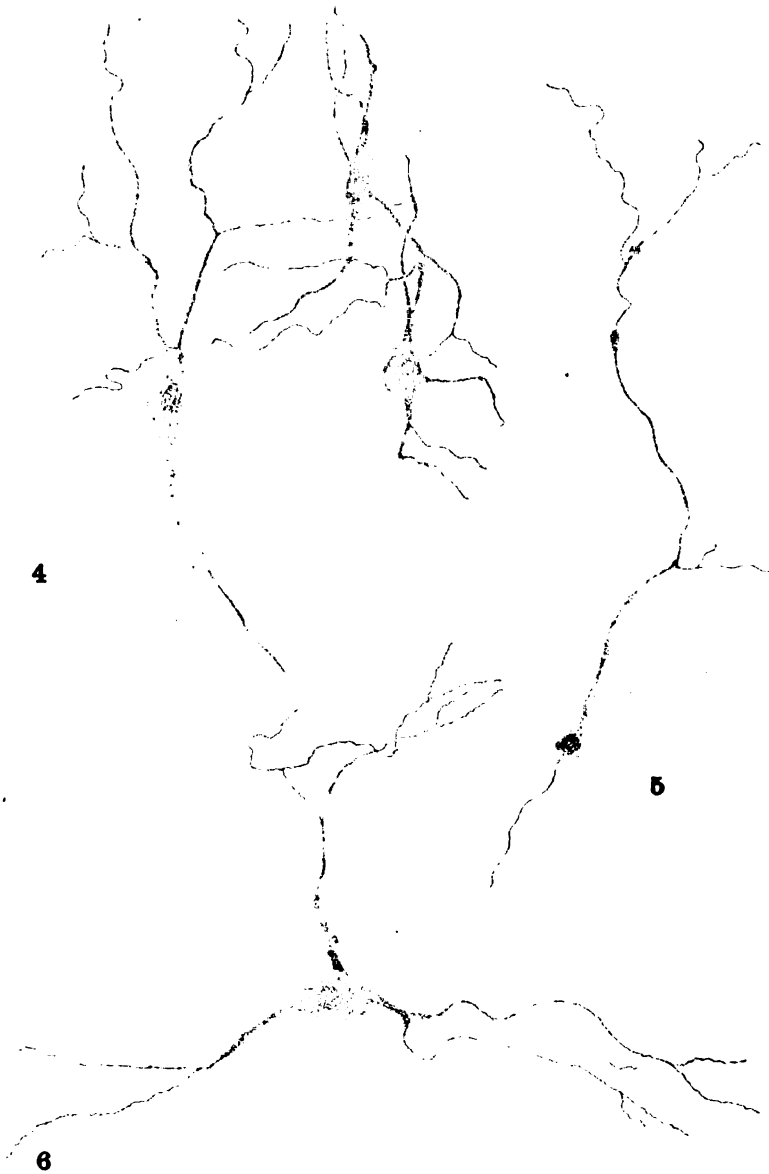
1. Drawing, made with the aid of the camera lucida, of the ganglion of the N. terminalis of *Mustelus canis* after staining with methylene blue (intra-vitam). The arrow points towards the peripheral end of the nerve. $\times 90$.

2, 3, 4, 5, 6. Drawings, made with the aid of the camera lucida, of cells occurring in the ganglion of the N. terminalis of *Mustelus canis* after staining with methylene blue (intra-vitam). The stipple is used in a conventional way and does not indicate protoplasmic granulations. Figures 2, 3, 4, 5, $\times 235$; figure 6, $\times 250$.



2

3



ON A LAW OF SPECIES IDENTITY OF THE NUCLEUS- PLASMA NORM FOR NERVE CELL BODIES OF CORRESPONDING TYPE

THE NUMERICAL CONSTANCY OF THE NUCLEUS-PLASMA COEFFI- CIENT OF THE FUNCTIONALLY RESTING PURKINJE CELL OF THE DOG SPECIES

DAVID H. DOLLEY

From the Pathological Laboratory of the University of Missouri

SIX FIGURES (ONE PLATE)

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INTRODUCTION

In an earlier paper ('10), the writer called attention to the fact that the nucleus-plasma coefficient of the resting Purkinje cell was a close numerical constant in three dogs, one being an undisturbed normal animal, the second having been exercised

in a treadmill, and the third irritated to a state of surgical shock. That is, in its simplest statement, the cell body of the average cell was exactly the same number of times larger than the nucleus in those cells which had remained in a condition of functional rest, whatever the degree of activity in other cells may have been or however the activity was induced. Further, in those extensive measurements it appeared that the coefficient figures of the nucleus-plasma relation for cells which were excited in the progress of activity to shifts above or below the level of the starting point, the resting cell, were of a denomination to warrant the opinion that they were based on the primary figure of the resting cell.

Subsequent measurements by several of my students on the rabbit gave additional data in support. The nucleus-plasma relation for the resting Purkinje cell has been found to be practically the same in all rabbits measured, the actual figure of course being different from that of the dog species for that cell. Further, in the single human case which has been studied there was the same evidence of the shifts of activity being based on a constant resting cell level ('11 b).

In the study of functional activity in the crayfish, *Cambarus virilis*, which followed ('13 a), not only was there the same finding for corresponding resting cells of the same type in all animals, but, totally unexpectedly, the nucleus-plasma coefficient of all types of resting cells, of which there are four, namely, central and centro-peripheral motor and central and centro-peripheral sensory, was an identical constant. In other words, in such a primitive animal, all cells are on the same plane as regards their nucleus-plasma relation. This is particularly surprising when it is considered that the volume of the largest resting cell, the central motor, is fully four times that of the smallest resting cell, the centro-peripheral sensory. Again the subsequent shifts resulting from activity are based on this common figure, as in the Purkinje cell. For this species of the crayfish, the number of animals from which few or many measurements were made, including both seventeen adults and fifteen new-born, was so great as to exclude variations innate or ac-

quired by disease and the technical results ran so smoothly, that the following definite conclusion was stated: "For resting cells of both sensory and motor types, in *Cambarus virilis*, there is a definite relation of nuclear mass to cell mass. The coefficient of this relation is an identical constant among the four types of primal resting cells both in the same animal and for all animals of the species, whatever their size." These facts, taken in connection with the constancy of the results for the Purkinje cell as representing adequately the highest type of specialization in animals in rest and activity toward the other extreme of the scale, appeared sufficient for the further generalization that for all nerve cells there is a certain definite relation of nuclear mass to cell mass, which is the primal principle of Richard Hertwig's nucleus-plasma relation theory ('03).

For that phase of the relation which interests us here, namely, whether the expression of this relation is an identical constant for corresponding cells in all animals belonging to any particular species, I have felt that the induction, while reasonably certain for the crayfish, was not sufficiently supported for other animals, though all the facts pointed that way. It must be pointed out here for the sake of a clear initial understanding that this phase of species identity of the relation is not at all carried by Hertwig's original statement above, nor is it necessary in the application of the nucleus-plasma doctrine in the explanation of the mechanics of function. Provided that the resting cell of any type of any animal have a nucleus-plasma relation which is a constant for that animal, "eine bestimmte Korrelation," the shifts and final upset which occur as a result of functional activity and functional depression could perfectly well be explained in terms of that animal's particular relation for that cell. There could as well be a general correspondence in the changes of function and of lack of function ontogenetically and phylogenetically that actually appears more constant and exact. The trend could be the same without such close quantitative uniformity as that to which the mass relations invariably point. It is needless to say though that the interpretation of the changes of function in all its phases on this basis would not harmonize so

well among different individuals as to have the exactitude of truth.

Accordingly, for the sake in part of supplying for the dog this deficiency of data, the present series of measurements was undertaken. Another and more immediate reason was that in a study of the development of function in the dog which has recently been carried on by Mr. Albert L. Jones and myself, the progress of the nucleus-plasma relation from the embryonic condition to that characteristic of full development has been consistently in harmony with a law of constant correspondence of size relation for the resting mature Purkinje cell. Consequently, it seemed advisable to make additional measurements from a wide assortment of animals, in order, if it continued to work out in harmony, to make the induction more sure and as well to eliminate reasonably the possibility of variations due to differences in age, size, breed, or accidental causes other than those which a priori should affect the constancy of the relation.

The species identity will be shown to hold for the resting Purkinje cell of the dog as well as for all resting cells of *Cambarus virilis*. The mechanism of function in both is identical ('13 a), the reaction of function is a purely quantitative one ('13 b), and the cell recovers to its prior state of constancy after function ('11 a). The actual figures from measurements of the stages of activity have corresponded so closely among different individuals as to lead naturally to the induction that members of a species must be to a remarkable degree on the same plane as concerns the quantitative relation of nucleus to plasma. There was therefore considerable collateral evidence pointing to a law of species identity, from the study of functional activity originally and of the development of function later, before the facts to be presented were sought. As a matter of logic, therefore, the present induction of a law is not based on a relatively few cases of coincidence, but the ground could be defended that these cases represent a verification, the final step in inductive logic.

The evidence again from all sources indicates that there is the same unity of mechanism for all other nerve cells ('11 b);

their reaction must be quantitative as well; therefore, the law can not be conceived otherwise than of universal application to nerve cells, type by type. At least that it holds for such wide extremes of animal life justifies a formal statement, and, until the exceptions be satisfactorily proved, one must follow the lead which is offered. This paper is written with the hope that the plain bearing of the results on obscure problems in biology will stimulate work by others. Many facts are needed and it will take a multitude of investigators to gather them.

SOURCE OF MATERIAL

The data of age and size of the animals are set forth in table 1 along with the measurements of cells, since it is necessary to consider their correlation closely. Resulting partly from the convenience of using material on hand and partly from choice limited by circumstance, a more heterogeneous assortment of animals could hardly be gathered together. The single condition of ineligibility imposed was the frank existence of functional depression. It is felt that the wide range of material goes far in offsetting what is in relation to a whole species a scant number of individuals. The material includes dogs of all weights from 1 kgm. to 20; of all ages from six weeks to obvious old age; of both sexes; of different grades of nutrition; of the most mixed breeds, though in a few one strain strongly predominated; and, finally and most important, of a wide range of functional states. For the sake of the emphasis proper for function, there may be specified: six normal animals showing various grades of activity from every-day life; two animals in constitution so weak that they succumbed to a few whiffs of an anesthetic; an animal exercised vigorously in a treadmill; an animal in which similar physical exercise preceded the shock of an hour's surgical operation whose final feature was complete exsanguination; three animals dying by accident in the course of different surgical operations; an animal some four weeks after a craniotomy whose purpose was to resect certain cerebellar fibers; and lastly an animal which received a large dose of lactic acid, a dose however ineffectual in producing frank anatomical depression.

There were thus all grades of activity from many kinds of stimuli, spontaneous, mechanical, chemical and trophic—alone and in various combinations. Yet there was activity and only activity and no depression anatomically apparent. Why the sole effort was directed toward the exclusion of depression will be clear from the later discussion. For the present purpose, any degree of activity whatever was found to make no difference so long as there were enough cells left at rest to measure.

The one possible discrepancy in the estimation of these experiments as uncomplicated by depression as a result of the various operative procedures, as I see it, will be frankly stated. This is the factor of ether anesthesia. While undoubtedly initially exciting, the essential effect of ether must be regarded by analogy in the present state of our knowledge as depressant. Yet in the ordinary anesthesia of experiment, however a relatively short one, there has never been any anatomical indication of frank depression, but on the contrary what effect there might have been was only part of a general excitation. Nevertheless such an effect of depression would reasonably be expected on physiological grounds to have appeared at some time, a lack of harmony that I am unable to explain. However, it has been the invariable finding for various types of depressants that they lead slowly to frank anatomical exhibition. This lets out the ether here as a theoretical factor of disturbance of the quantitative status of the resting cell as such. So far, a single large dose in the case of the waste products of fatigue and in the case of morphine has been ineffectual, save to an almost negligible degree ('14). Repeated and long administrations are required. So the ether is following what appears to be a general rule. This is in direct contrast to activity in which even a small excitatory stimulation produces a measurable result ('09). It is entirely possible that depressant agents will be shown to accomplish their milder physiological and symptomatic results, their ordinary result as we use them, by hindering intracellular coordination in the same way that has been demonstrated for frank depression ('13 b) and yet to act too superficially to disturb the organic constitution visibly. We have no idea, speaking from the point

of view of the cell, how much depression it takes to produce even a symptom, to say nothing of actual unconsciousness. On the possibility of affinities of localization for such ordinary depressants, while not to be neglected, I look with some skepticism, for one reason, because in all the depressants studied the cerebellum does become involved with repeated administration. At any rate, the belief is based on abundant facts that ether may be disregarded in the present connection as an active, anatomically demonstrated depressant which might affect the resting cell, and the uniformity of the results confirms its correctness. As a matter of fact, the ether factor is not involved in many of the present experiments. Outside of the operative cases, the animals were killed in various ways—by chloroform, by illuminating gas, and by stabbing after an initial nitrous oxide anesthesia.

The essential characteristics of each animal follow in the order of the table:

Experiment Normal 13. A mongrel male pup, the best grown of his litter.

Experiment Normal 12. A nursing mongrel male pup, well nourished and just able to walk well, whose cells showed a high degree of activity.

Experiment Development 19. A well nourished and developed female pup of pointer and bull strains, the mother being of pure breed.

Experiment Muscular Exertion 30a. A female pup of shepherd strain which died during etherization at the start of a lactic acid injection and was used as a control. An extremely emaciated animal which showed a marked degree of functional activity (See count in table 1, 1914).

Experiment Anesthetic 1. A lean mongrel, sex not noted. Cardiac and respiratory failure occurred after a minimum of ether and efforts at resuscitation were totally ineffectual. In connection with this example of occasionally extreme susceptibility, it may be noted that the animal showed the nearest approach to cellular exhaustion that has ever been observed in an experimentally undisturbed animal, more pronounced even than the usual profound shock condition.

Experiment Normal 10. A fat female shepherd mongrel.

Experiment Muscular Exertion 5. A well nourished male hound pup of fairly pure strain. The control animal of the first muscular-exertion series (1909).

Experimental Muscular Exertion 3. A male from the same litter as Experiment Muscular-Exertion 5. An extremely strong and active animal which was exercised in the treadmill for one hour, one-fourth of which time was distributed as periods of rest.

Experiment Muscular-Exertion 32. A young adult female hound. The animal was exercised in the treadmill for two hours, of which time somewhat less than half was devoted to rest, and then became the donor in a repetition of Mosso's experiment of the transfusion of the blood of fatigue (1914), in which it was well exsanguinated. The total time of anesthesia before and after operation was one hour and ten minutes. The dog was in good training and stood the strain remarkably.

Experiment Shock 36. A well nourished mongrel female, which was probably half-grown. Killed by accident by a stab into the medulla during an operation which will be described under Experiment Cerebellar Section 4. The operation had lasted probably some thirty minutes.

Experiment Shock 37. A well nourished and probably nearly grown male pup, whose mother was a well-bred pointer. Died suddenly of cardiac embolism in an experiment of introducing seeds into the arterial circulation after an anesthesia and operation of forty minutes.

Experiment Cerebellar Section 4. A very old mongrel male dog, well nourished. The animal was killed twenty-seven days after a craniotomy and a stab by a thin two-edged spear-like knife into the worm of the cerebellum parallel to its surface and at a depth of some five millimeters. The purpose of this was to sever the axones of Purkinje cells within a localized area and has been a phase of the correlation and identification of the effects of axone resection with functional depression within their physiological limits. At the time of death, the animal was outwardly recovered, and, cytologically, outside of depression and degeneration within the limited area of section, the remaining parts of the cerebellum showed only the normal changes of activity with a smaller number than usual of cells at rest. That is, whatever effect the various factors of operation and anesthetic may have had were limited to functional excitation. The sections from which the present measurements were made were taken from a lateral lobe. There were many cells of senile type which offer no conflict with the above analysis.

Experiment Shock 38. A young adult male mongrel. Died from a badly handled anesthesia in the course of an operation of cerebellar section. The operation had lasted probably thirty minutes.

Experimental Normal 19. A fairly pure bred pointer, which had given birth three weeks prior to death. A lean muscular animal of large build.

Experimental Muscular-Exertion 31 (1914). A female collie, showing many cells of senile type. Experimentally, the animal had received intravenously under ether 350 cc. of a 5 per cent solution of lactic acid in warm saline, a total of 17.5 cc. of the full strength acid. She showed the usual symptoms of apathy and sleepiness and vomited. At the time of killing, which was after five and one-half hours, the symptoms were still preceptible though less marked. This animal was chosen purposely to see if the measurements confirmed the ob-

jective anatomical diagnosis of the absence of frank functional depression after a single large and symptom-producing dose. Such variations as were found went with the obvious degree of senility of the cells measured (table 5).

Experiment Normal 18 (table 5). A muscular and well nourished female, of predominant bull strain. It had given birth to pups three weeks prior. This animal, presumed to be a normal, exhibited frank anatomical depression and was used for measurements of cells in that state.

TECHNICAL METHODS

Only an outline necessary to clear understanding is inserted here. The factors which might lead to error are most appropriately summarized under "Technical factors in their relation to the results," because their relative importance will be plainer after the essential results are stated. Further, as the tedious data in this section to follow are only contributory to the main theme, that section may be omitted unless the possible interest is in criticism or repetition.

I desire to express my thanks to my colleagues in Mathematics, Prof. E. R. Hedrick and Prof. O. D. Kellogg, for their painstaking elucidation at many times of various mathematical principles involved. However, they are not to be held responsible for my possibly anomalous manner of discussion of an unfamiliar subject.

1. The calculation for aggregate cells from the average of measurements

The technic of measurement and the principles of calculation have been previously discussed in sufficient detail ('10, '13 a). Each set in tables 1 and 5 represents the average of twenty-five resting cells. It is very seldom that this number has not been found amply sufficient to give a fair average among the different stages of activity. Ten cells give a constant relation more frequently than they do not. The measurements were made on camera lucida projections. The longitudinal and transverse diameters of both cells and nuclei were ascertained in terms of the half-millimeter as a unit, an average was taken, and the figures reduced to micra. The long diameters multiplied by

the square of the short diameters gave an expression of the relative volumes which may be proved mathematically to be as exact as actual calculations of volume. The same lens system was used throughout, Zeiss comp. oc. 8, 2 mm. oil immersion, normal tube length, $\times 1960$ at table level.

2. The calculation for the individual cell from serial sections

a. By wax reconstruction. Sections in serial were cut at two and at one micra from two animals, Experiments Shock 37 and Normal 19. The Minot precision microtome was employed. The one micron sections were only obtained by lightly bevelling an extremely sharp and microscopically smooth edge of the knife in the fashion of a scissors blade. With sufficient perseverance places were found which gave fairly perfect sections, free from side to side compression and wrinkling. Dusting the knife surface with talcum powder helped greatly to prevent wrinkling and sticking. Fortunately with such an edge the sections ribbon nicely.

The knife set for two micra gave from seven to eleven sections through individual resting cells and for one micron from sixteen to twenty-three, which is only one of many confirmations of accuracy on the mechanical side.

The adjustment of the magnification to correspond to the thickness of the wax plate was the step next in place and importance. After making a plate of approximately the desired thickness, its exact thickness was determined by the use of a micrometer caliper reading accurately to hundredths and closely to thousandths. Twelve readings were made at different places on the plate and the average reading was used as the working figure. No plate used gave an extreme of variation in the individual readings of more than one-tenth of a millimeter. So great a variation was very exceptional. The determination of the magnification to suit was also made to depend upon the average of numerous readings from the Zeiss object-micrometer. Various combinations of lenses and tube length were necessary and all projections were drawn at stage level. The majority of recon-

structions were made from two plates, so that the factors of possible variation involved in the thickness of the plates and the magnification are constant within each set and are shown to be of very minor importance (table 2).

In other respects the technical procedures were the usual ones. Sixteen cells were reconstructed, eleven from two micra serials and five from one micron, with one repetition. Number one was a trial cell for various points of technic and hence is not included. Figures 1 to 5 illustrate the various types of cells.

When each cell was completed to the point of being ready to have the straight edges of its component layers smoothed down to a curved surface, the plasma and the nucleus were weighed separately on the theory that the quantitative relations of their prototypes would be preserved.

b. *By application of the prismoid formulas.* The data obtained for wax reconstruction are suitable for the application of Simpson's Rule or some form of the Simpson-Lagrange Approximation derived therefrom to determine the volume. No mathematical discussion will be attempted and for the statement of the formulas the reader is referred to any standard work on calculus (in the calculus by Davis-Hedrick on pages 129, 240 and tables 45). The area of each layer can be exactly determined, and the thickness of the layer, the number of layers, and the number of planes of section are known. The area was found by using a polar planimeter on the original camera lucida tracings. The number of planes of section is one greater than the number of layers, that is, the outermost extremity of one end layer was counted as one 0 area, while the estimated area of the opposite extreme end layer, usually of very diminished size, was taken as the closest approximation of the other 0 area. The actual procedure, while tedious, is very simple.

The results in the case of the one micron sections are so consistent with those obtained by weighing the wax models that there is every indication of exactness (table 3). The results in the case of the two micra sections are more variable, which will be explained in the section on "Technical factors." By way of further checking up the results, since the mathematically

obtained figures representing volume are larger than the figures from wax reconstruction representing weight, the volume of one wax cell (Number 9) was calculated by weighing the water displacement. While rather crudely done, the volume of the plasma came within two cubic centimeters of the volume figure in table 3, and the volume of the nucleus corresponded more closely (92.7 to 7.7). It seemed sufficient.

THE NUMERICAL STATEMENT FOR THE DOG OF THE SPECIES CONSTANCY OF THE NUCLEUS-PLASMA COEFFICIENT OF THE FUNCTIONALLY RESTING PURKINJE CELL

This statement is very simple. The nucleus-plasma coefficient is the figure obtained by dividing the nuclear mass into the plasmic mass, the plasmic mass being the total cell volume less the nuclear volume. In the case of the wax reconstruction the plasma and nucleus are of course separately constructed. The figures for the coefficients are placed in the last columns of tables 1, 2 and 3.

First, after the method of average measurements, the great majority of the figures for the resting Purkinje cell are in the close neighborhood of eleven (table 1). The only noteworthy exceptions are three, all on the maximum side and barely over twelve. These exceptions are regarded as well within the limits of permissible technical variation, for definite reasons to be given under that section, and are so explained, even without calling upon a biological factor of variation which is possible in one of them. (Senility, Experiment Cerebellar Section 4). As a matter of fact, the greatest exception, Experiment Shock 37, was for that reason one chosen for the wax reconstruction. By this method, away from the limitations of average measurements, it is brought into line of constancy.

Second, after wax reconstruction, the figures from the more impersonal and mechanical and hence more reliable one micron serials are within the range of the most uniform of the preceding results (table 2). The arithmetic mean of the coefficients of the five cells is 11.129, the standard deviation is $\pm .2037$, and the coefficient of variation is .01830.

TABLE 1
Dimensions, relative volumes and nucleus-plasma coefficients of functionally resting normal Purkinje cells of fifteen members of the dog species

NUMBER OF EXPERIMENT	AGE	WEIGHT IN GRAMS	DIAMETERS OF AVERAGE CELL IN MICRA	DIAMETERS OF AVERAGE NUCLEUS IN MICRA	RELATIVE VOLUME OF CELL	RELATIVE VOLUME OF PLASMA	RELATIVE VOLUME OF NUCLEUS	NUCLEUS-PLASMA COEFFICIENT
Normal 13.....	40 days	980	32.61×16.74	11.21×8.22	9138	8381	757	11.07
Normal 12.....	42 days	995	34.61×17.60	11.52×8.81	10720	9826	894	10.99
Development 19.....	42 days	3555	37.70×17.60	12.80×8.72	11677	10704	973	11.00
Muscular-exertion 30a.....	60 days	1240	35.51×16.12	11.50×8.16	9227	8462	765	11.06
Anesthetic 1.....	3 mo. ±	3080	37.04×16.78	12.53×8.12	10411	9584	827	11.58
Normal 10.....	4-5 mo.	5700	37.84×17.62	12.68×8.77	11747	10772	975	11.04
Muscular-exertion 5 ¹	5 mo.	5900	31.85×18.46	11.21×8.60	10853	10024	829	12.09
Muscular-exertion 3 ¹	5 mo.	7200	31.66×19.41	12.57×8.67	11927	10983	944	11.63
Muscular-exertion 32.....	young adult	8000	32.35×17.73	11.87×8.43	10169	9326	843	11.06
Shock 36.....	not fully grown	8870	40.43×17.50	14.65×8.01	12497	11527	970	11.88
Shock 37.....	not fully grown	10620	40.34×17.19	13.62×8.09	11920	11029	891	12.37
Cerebellar section 4.....	senile	13240	38.36×17.90	13.69×8.27	12280	11354	936	12.13
Shock 38.....	adult	13400	36.37×17.85	13.07×8.40	11588	10666	922	11.59
Normal 19.....	old	19215	40.84×21.17	13.55×10.44	18303	16826	1477	11.32
Muscular-exertion 31.....	senile	19570	35.34×18.73	11.94×9.21	12397	11384	1013	11.23

¹ Repeated from Tables 1 and 3, pp. 363, 367, Jour. Med. Research, vol. 22, 1910. Weight originally in pounds.

Yet one animal was in shock, and one was double the size of the other. It is true there are only five examples but it was felt at the beginning of this final test that to obtain even one successful result would be conclusive, and more than that, two of the five cells—eleven (figure 1) and fifteen—are so peculiar and atypical in their shape, though corresponding in other respects to the resting type that no test could be more rigorous. The most convincing point in the wax reconstruction was that so far as one could judge the possibility of a closer approximation to exactness in any cell than was ventured, it would have tended to lessen the deviation from a constant mean.

TABLE 2
Weights and nucleus-plasma coefficients of wax models

NUMBER OF EXPERIMENT	NUMBER OF CELL	THICKNESS OF WAX PLATE IN MM.	MAGNIFICATION	WEIGHT OF WAX PLASMA IN GRAMS	WEIGHT OF WAX NUCLEUS	NUCLEUS-PLASMA COEFFICIENT
Experiment Shock 37	<i>After two-micra serials</i>					
	2	4.970	2500	78.65	6.95	11.320
	3	5.683	2840	130.20	11.91	10.932
	4	6.287	3150	167.60	13.19	12.706
	4 (repeated)	2.182	2180	55.25	4.76	11.607
	5	5.683	2840	127.90	10.91	11.723
	6	5.683	2840	135.71	12.79	10.610
	7	5.683	2840	120.40	10.38	11.599
	8	5.683	2840	136.41	12.70	10.741
	<i>After one-micron serials</i>					
	9	2.560	2560	81.50	7.40	11.013
	10	2.560	2560	78.90	6.92	11.401
	11	2.560	2560	63.29	5.64	11.221
	16	2.560	2560	98.32	9.10	10.804
Experiment Normal 19	<i>After two-micra serials</i>					
	12	2.182	2180	48.02	4.43	10.839
	13	2.182	2180	54.51	5.12	10.646
	14	5.189	2590	94.71	8.90	10.641
	<i>After one-micron serial</i>					
	15	2.560	2560	48.30	4.31	11.206

These five cells in one micron serials carry with them the approximate correctness of the results from the more nonobjective two micra serials. In the two micra serials the range of variation is practically within one unit, if the one exception (cell 4) be excluded which disappeared when the technical cause was eliminated (see section on "Technical variations"). The application to the aggregate is thus broadened in a sufficiently conclusive way when compared with individual cell results making up any of the series of twenty-five cells averaged in table 1. In none of the eight series for which the statistical data will be given later were there as many as ten cells so close to a constant mean. A glance at the frequency-polygon will make this more clear (text-fig. A).

Third, after mathematical computation from the data of serial sections, the figure for each of the one-micron cells is very slightly larger than the corresponding figure from wax

TABLE 3

Volumes and nucleus-plasma coefficients from prismoid formulas

NUMBER OF CELL (Table 2)	VOLUME OF CELL IN CC.	VOLUME OF NUCLEUS IN CC.	VOLUME OF PLASMA	NUCLEUS- PLASMA CO- EFFICIENT	REMARKS
<i>After two-micra serials</i>					
2	82.506	5.835	76.671	13.138	Cell and nucleus each had one end section of partial depth.
4 (first)	193.452	13.075	180.377	13.795	Cell with one, nucleus with two end sections of partial depth
8	158.572	14.029	144.543	10.303	All sections complete throughout
<i>After one-micron serials</i>					
9	94.651	7.766	86.885	11.187	Transition stage from rest to activity.
10	89.198	7.093	82.105	11.575	
11	75.325	6.056	69.269	11.438	
16	111.726	9.337	102.389	10.965	
15	54.920	4.539	50.381	11.099	

reconstruction. It was predicted by Professor Hedrick from the nature of the data that such would be the case. For the two-micra cells it will only be said here that Cell 8 which approximates the result from the wax reconstruction most closely was the most suitable for application of the prismoid formula in that the polar sections of both plasma and nucleus were apparently of full thickness.

The results are regarded as so consistent, when all possible factors of variation are considered, that a law is formulated as follows:

Resting undepressed nerve cells of corresponding type of all individuals of a species have a mass relation of nucleus to plasma which is a close numerical constant, whatever the age between full development of the relation and senescence, whatever the size of the animal, irrespective of variations in the dimensions and absolute size of the cells both in the same animal and in different animals, and irrespective of the degree of function in excited cells of the same type in the same animal.

Certain points in table 1 in regard to comparative sizes and relations of cells may be conveniently considered here. Only the adult animals, from Experiment Muscular Exertion 32 to the end, excepting Experiments Shock 36 and 37, can enter into the comparison. Since the other animals were yet in progress of growth, analysis is not permitted. In the first place, there is no definite correspondence of size of cell to size of animal (see column of cell volumes). The cells of the two largest animals, Experiments Normal 19 and Muscular Exertion 31, which are practically of the same weight, differ widely in size. While Experiment Muscular Exertion 31 is two and one-half times larger than Experiment Muscular Exertion 32, its cells are only about 20 per cent larger.

Levi ('05) and Conklin ('12 b) conclude that the size of the nerve cell varies with the size of the animal. If there is any such general rule, these data are sufficient to bring out a striking exception. But in such comparisons the effect of function on the size of the cell has been disregarded (see under "Technical factors"), and resting cells afford the only exact basis of com-

parison. When the factor of function is reckoned with, it is probable that the nerve cell is not the exception it appeared to be to Conklin's conclusion that within the same species cell size is approximately constant.

Since the data are meager, they were also examined mathematically to see if there was anywhere a definite ratio of cell size to body size with a progressive increase of body size. Experiment Normal 18 (first and fourth series in table 5) was included. By comparison of the ratio of body weight to body weight and of cell size to cell size between Experiment Muscular Exertion 32 and Experiments Normal 18 and Normal 19 respectively, the coefficient factors for body size are 2.4 and for cell size 1.8 in the one ($1 : 2.4 = 1.27 : 2.29$) and 1.93 and 1.31 in the other ($1 : 1.93 = 1.27 : 1.67$), which gives a disproportionately larger size of cell in the smaller animal. Between Experiments Cerebellar Section 4 and Normal 19, the coefficient factors are 1.45 and 1.48 ($2.2 : 3.2 = 2.05 : 3.05$), in which the cells maintain practically the same ratio as the body weight. Between Normal 18 and Normal 19, the coefficient factors are 1.25 and 1.36 ($2.57 : 3.2 = 2.24 : 3.05$), which is in slight degree the reverse of the first result.

A priori, I feel as certain as one can that no exact correspondence of nerve cell size to body size will be found. The unpublished study of the development of function has molded the opinion that it is the factor of individual function in the nerve cell which fundamentally determines the size of its resting cell and nucleus. Its principal growth is functional growth. Primarily, size will vary according to function. On this basis, the striking exception to any correspondence exhibited by the Purkinje cells of Experiment Muscular Exertion 31 is readily explained. Some further discussion is connected with the section on "Significance of the constant nucleus-plasma relation."

It must be distinctly understood that this lack of correspondence does not disturb the fixity of the relation between the plasma and the nucleus. Whatever the absolute size of the cell, that is constant. Certain points will emphasize how variations in one element of the cell are always complemented in the

other to declare a fixed relation: the most elongated cells, namely, with the longest major axes, are complemented by a similar shape of the nucleus (Experiments Normal 10, Cerebellar Section 4, Shock 36 and 37, and Normal 19); second, the shortest cells are equalized by a comparatively longer transverse diameter (Experiments Muscular Exertion 5 and 3).

THE MORE EXACT DEFINITION OF THE RESTING CELL RESULTING
FROM THE SPECIES IDENTITY

The resting Purkinje cell is a distinct type, recognizable from its shape, its content and arrangement of substance, its staining reaction, and the absence of certain structural alterations characteristic of either activity or depression. It was the last point, of absence of indubitable functional changes, that gave the first morphological clue to its identity. From youth to its prime the contour of cell body and nucleus is even and regular. It shows at most here and there a waviness of outline, but not that crenation and shrinkage which is so prominent in the Hodge stage of activity and which becomes even more pronounced in senility, in short, in temporary and final fatigue. This emphasis of its regularity does not exclude unimportant eccentricities of shape common to all cells of its type whether active or not. The pear-shape may be somewhat attenuated or it may be stout, the long axis may be curved this way or that, or there may be two separate dendrites coming off from the cell body. It is, however, more uniformly regular as a rule than any cell with which it might be confused in diagnosis and more important it shows none of the functional changes of irregularity.

The nucleus in a youthful or virile animal is packed with nucleolar substance and hence takes a deeper tinge of the acid stain. Its mesh is so close that with the net-knots it has a granular or pebbled surface appearance. It is uniform, without rifts or vacuolation from edema. The abundance of nucleolar substance and the absence of edema distinguish it sharply from the later stages of activity which are characterized by the loss of this substance and the advancing presence of edema. Outside of the karyosome it has no basichromatin and the presence

of any removes it from the resting class and marks the beginning hyperchromatism of early activity. The extra-nuclear chromatin, the Nissl substance, is uniformly distributed in the manner described in such detail by Nissl, usually also prominently within the dendrite. However, it must be emphasized that the amount of chromatin—and this applies also to the nucleolar substance—is variable and depends on the individual within limits that do not encroach on the frank hyperchromatism of early or the hypochromatism of later activity. The first thing in analyzing cell states is to make a survey to determine the standard of chromatin, at the least whether it inclines to an excess or a deficiency. This individual standard will run throughout the functional range. Again, in the resting cell's plasma there is the total absence of functional edema or vacuolation, but instead the plasma has a solidity of look and elects a deeper stain than the active stages outside of hyperchromatism itself, which has this more compact appearance of the "unstainable" substance intensified where the excess of chromatin does not obscure it. In short, the resting cell has throughout a look of preparedness.

Finally, the complex of absolute size and relative size of nucleus to plasma in the resting cell are sufficiently individual so that after experience from measuring one can recognize it from that alone amid the range of shifting sizes when it is modified by functional depression.

This detail has been given to show that the objective diagnosis of the resting cell is easy, after experience unmistakable from the time of the disappearance of any vestige of the embryonic state through its prime and maturity. Difficulty is found only at the transition point from the embryonic condition and with the onset of its old age—and its old age does not necessarily mean the old age of the animal (Dolley '11 a). In the former case, distinct stages of activity are superimposed before the nucleolar substance has accumulated to its full growth—its lack being an embryonic characteristic. Hence the undeveloped resting cell has a nucleus closely resembling the transition from the Hodge stage to nuclear hypertrophy (nuclear edema, late Hodge stage,

5" in all publications). . This applies to young dogs from ten days to five weeks, becoming progressively less marked. I was fooled by it until the incongruity of the measurements showed there was something wrong. The cytoplasmic edema is the clue in such cases. Edema is absent in the resting cell, but present in the confusing one.

Since so much is to be said about the factor of senility, its resting type demands a word. In frank old age without actual loss of cell organelles there is an appreciable deficiency of everything, of chromatin and nucleolar substance in particular, though the features of the virile cell are in the main preserved. There is a resultant shrinkage and irregularity of the cell and nucleus whose degree depends on the degree of senility. The difficulty in old age comes in differentiation from that same stage of activity as in early youth. This is due to the fact that the resting nucleus of age may be so deficient that it has the reticulated structure of the above transition. Here also in a declining hyperchromatism the cell comes to a semblance of the normal resting extra-nuclear content. Further, the resting cell is irregular from age and smaller than in youth, while the confused cell is also irregular, though usually more so, and small, that being its nature as a Hodge stage. Even with experience the diagnosis is frequently impossible, though it is not a practical matter as other cells less changed are at one's disposal.

This is the cell, so characteristically individual, that is regarded as one in functional rest, though in 'tone' and prepared for work. Earlier measurements confirmed its place. Additional data predicate that in one phase of its size relations there is the constancy of a law. Holding to that law as sufficiently established, one may add to the objective definitive points enumerated for the resting cell of the dog species the measured property of a nucleus-plasma coefficient constant for the species. It is true that at one transition point of activity an identical figure is reached and will be reached in every nerve cell above a certain point of differentiation. This is in passing from the maximum of upset in favor of the nucleus (earlier nuclear hypertrophy) to the final reversal in favor of the cytoplasm (ensuing cytoplas-

mic hypertrophy) which brings the coefficient figure from below eleven to above eleven ('10). There is no chance of confusion for the functioning cell is much larger and there is marked edema, paleness, and loss of substance. Morphologically, until exception be proved, the dog's resting Purkinje cell is fully defined.

THE TECHNICAL FACTORS IN THEIR RELATION TO THE RESULTS

1. *In the method of average of measurements*

In order to compare the different series, the usual statistical data were computed in eight experiments, as set forth in table 4. These series are well scattered and undoubtedly represent adequately the whole. The dispersion of the coefficients of the

TABLE 4
Statistical data from individual cell measurements

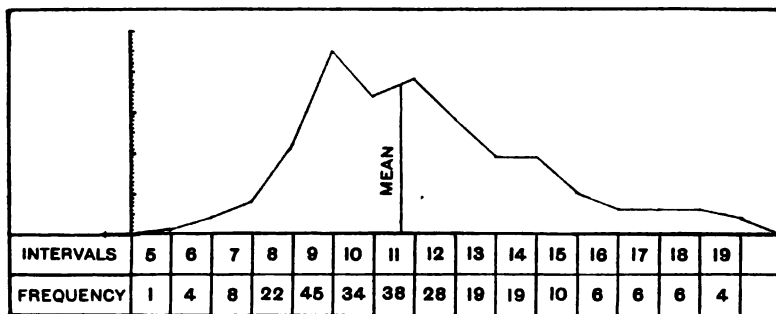
NUMBER OF EXPERIMENT	AVERAGE OF INDIVIDUAL COEFFICIENTS ¹	AVERAGE DEVIATION	STANDARD DEVIATION	COEFFICIENT OF VARIATION	PROBABLE ERROR	STANDARD DEVIATION OF THE AVERAGE
Normal 13.....	10.94	2.25	2.705	0.2443	0.3649	0.541
Normal 12.....	11.01	2.22	2.967	0.2609	0.3999	0.593
Muscular-exertion 30a..	11.30	2.23	2.938	0.2671	0.3959	0.587
Anesthetic 1.....	11.91	2.44	2.872	0.2597	0.3872	0.574
Muscular-exertion 32..	11.45	2.10	2.716	0.2456	0.3662	0.543
Shock 36.....	12.41					
Shock 37.....	12.61	1.58	2.038	0.1634	0.2745	0.407
Cerebellar section 4....	12.56					
Normal 19.....	11.54	2.19	2.863	0.2529	0.3858	0.572
Muscular-exertion 31..	11.23	1.68	2.065	0.1839	0.2785	0.413

¹ Inserted for comparison. The statistical data are computed from the means in table 1.

individual cells is the only definite basis of comparison. In two series, the coefficient was actually computed. In the others, this figure was read off directly from the slide rule. Numerous tests never gave a difference from the actual computation of more than .03, so that this is sufficiently accurate. In addition, the coefficients were estimated in two other series (table 4) but no other data were computed. There were thus 250 cells for comparison. From these the frequency-distribution was tabu-

lated and the results are here represented in a graphic way by the frequency-polygon of text figure A.

It is sufficient to say that there is nothing to distinguish one series from another in the way the figures run. They are dispersed above or below the same median. Sixty-six per cent of all cells fall within two units of the common arithmetic mean. The tendency toward constancy is the more remarkable, outside of the numerous chances for variation which will be mentioned, because the length of the third diameter, the depth of the cell, must in such measurements as these be assumed to be equal



Text fig. A Frequency-polygon of 250 cells

to the transverse diameter of the cell section. Though this unknown diameter is probably fairly uniform, it is one of the factors of dispersion that must be smoothed out by averages. In the measurements of the crayfish for example, where the third dimension was actually approximated, though the transverse diameters of cells are frequently unequal, the standard deviation was represented by smaller figures, ± 1.3 and ± 1.65 .

The most important point in explanation of why the figures for the nucleus-plasma coefficients are so constant is as follows: The nucleus-plasma coefficient of the resting cell holds to the same figure throughout the first stage of activity when that resting cell is excited to activity. That is, though both cells and nuclei increase in size, they increase in exactly the same proportion. In the Purkinje cell this has been true for an in-

crease of as much as 50 per cent, for the crayfish cells for as much as one hundred per cent, namely to double the size of the starting point cell. This so far invariable result is held, in the first place, to give a wider basis for accepting the uniformity of relation among various sized resting cells. Cells even more variant in absolute size have an identical relation. Technically, it makes the selection of cells for measurement much simpler. To restrict one's self to a hard and fast type of resting cell would make the proposition very difficult, for one thing, for example, in the matter alone of finding them in highly excited animals such as were used. A somewhat broader range of selection which was postulated on the basis of uniform relation is permissible. Slight indications of beginning activity, a little more chromatin than usual for example in an otherwise conforming type, make no difference. As a matter of fact, a few cells midway between rest and the maximum of the first stage of activity were included in some experiments to see if the result was appreciably affected. It was not (compare also Cell 16, table 2). Still, since even within the strict resting type the cells do vary considerably in absolute size, possessing only in the matter of size the one common characteristic of nucleus-plasma ratio, the doubt involved was put to the following actual test of calculation in one series, in default of a mathematical investigation of the probabilities and the reasons therefor, which is beyond the writer's power.

It must be kept in mind that the routine method of calculation employed was to take the average of the diameters of the twenty-five cells in each series and from these average figures the volumes and coefficients were calculated. In Experiment Shock 37 which was chosen for a test of the accuracy of this, the figure for the coefficient thus obtained is 12.37 (table 1). Now, instead of using the diameters, the individual volumes of the cells and the nuclei of this series were calculated from the individual dimensions, and the volumes themselves averaged. The resulting coefficient figure was also 12.37. Finally, the average was taken of the series of coefficient figures obtained from each individual calculation and the figure was 12.61. These

computations were made by aid of the slide rule but are thought to be sufficiently exact for this comparison. There is nothing to distinguish Experiment Shock 37 from any of the other experiments in the matter of its range in size of individual cells. Finally, the average of the individual coefficients for a total of 250 cells (table 4) is 11.696. The mean of the average coefficient figures in table 1 for the same experiments is 11.469. These facts would seem sufficiently to answer a possible objection that the average of individual volumes should have been used rather than the average of individual dimensions. Practically, one comes to the same result whatever the method of averaging.

Considering further the possibilities of variation dependent upon the nature of the calculations, it will serve to emphasize the constancy of the coefficient figures to point out how small differences in diameter or volume figures are magnified in the resulting coefficient figures. Thus, as a single example, it would only take an increase in the nuclear volume of forty cubic micra in Experiment Shock 37, whose coefficient figure is the most widely variant by average measurements, namely 12.37, to make the resulting coefficient 11.82, which in any estimation of an average calculation would be a slight variation. It must be remembered also that here one is dealing with cubic contents. Small differences in the diameters, particularly the transverse diameters, become so much the more magnified. One micron less for the transverse diameter of Experiment Shock 37 would reduce the nucleus-plasma coefficient from 12.37 to 10.86.

Next, of the purely technical factors of variation, this same experiment actually illustrates what is probably, next to deviation from a diametral plane of section, the most important one. This is the determination of the longitudinal diameter of the cell body. The rule followed was to measure to the point where the dendrite becomes a constant size. When one has measured hundreds of cells, the determination of this becomes a fair constant for that measurer. Yet even then it occurs that certain dogs have exceptionally slender cells; there is no definite transition from cell to dendrite, but the change is most gradual, so that the measurement is an approximation. Experiment Shock

37 had cells of this type. The length of the long diameter thus exaggerated would explain the exceptional figure in table 1, and the wax reconstruction makes it certainly accidental. As a matter of fact, the individual computations in this series are the most uniform of all, as table 4 shows.

For the most part the cells selected belonged to the usual pear-shaped form. However, the more uncommon forms were not refused in all the series. The inclusion of cells with two dendrites or of cells broader than long, at least, does not affect greatly the resultant ratio when they are measured according to fixed rules, that is, their nuclei correspond. On the other hand, that it is not merely a matter of averages is indicated by the fact that when certain types of activity, as mentioned under "Definition of the resting cell," were confused with the resting cell and included, an inharmonious result was obtained. This was the case in Experiment Muscular Exertion 30a, for which the recorded measurements represent a corrected repetition.

It was the custom in making the measurements to record on any cell peculiarities of whatever nature that would render it questionable beyond the usual limits of acceptance. If more acceptable cells appeared in sufficient number in a reasonable number of sections—each series represents the survey of some twenty-four to forty sections—these questionable cells were then possibly disregarded. As the aim was rather to give every chance for variations to show up, the different series in by far the most part, to be exact, in 11 out of 15 series in table 1, represent the first twenty-five cells met that were diagnosed of the resting state and were sufficiently and properly in section. The latter is the main handicap. No averages were made until all cells were selected, all repetitions of measurements are noted in the text, and there is only one case in which a cell was excluded after the whole calculation was finished because the nucleus was obviously too small to be fully in section (Experiment Shock 36). The coefficient was thereby changed from 12.18 to 11.88. It also seems proper to say that the variations in size and shape and in relative size of cell to nucleus are sufficiently great to make it impossible to know how the measurements will result

and hence one dare not be influenced by any desired result. The necessity of consciously following fixed rules of measurement quickly becomes impressed.

Finally, the chief factor of variation is the nonmedian or non-diametral section of both cell body and nucleus. To this the following considerations apply.

1. The structural indications of a median section are fairly definite. However, as table 4 shows, the exactness of a median section or the lack of exactness was not made a preponderant requirement in the selection of a cell or in its rejection except beyond a very plain appearance of disproportion. Cells were chosen primarily because of certain objective characteristics as described in the last section, and it was only required that they show a fair approximation of a median section.

2. The diameter of the cross section of a nearly spherical body varies very slowly for plane sections nearly median or diametral.

3. The deviation from an actual diametral plane varies on both sides of a certain mean. Considering the numerous possibilities, not only of varying planes of section but of an eccentric position of the nucleus, the only hope of approximation to an average exactness lies in admitting cells whose elements it may be are obviously slightly out of relative proportion, one way or the other. No line could be drawn.

4. At any rate, the smallness of the probable error indicates that relative variations from the medial plane are probably not large.

The suggestion of these points is due to Prof. O. D. Kellogg of the Department of Mathematics.

2. In the method of wax reconstruction

Of the technical difficulties which it was found necessary to overcome as far as possible, only two need be discussed in detail as being especially important.

1. The possible incompleteness of end, that is, conjugate polar sections. This may apply to one or both poles of the nucleus or plasma or both. In the two micra series, only two cells came

out with the end sections of both nucleus and plasma apparently complete and without a trace of either element in the immediately adjoining sections in the four possible chances (Cells 8 and 12). Since a partial section estimated as a full one not only makes an appreciable difference in the weight but also makes a disproportionate difference as regards mass relations on account of the much smaller size of the nucleus, it was obviously necessary to attempt an approximation of the actual thickness. The reading of the optical section from the scale on the Zeiss fine adjustment screwhead helped materially, but no attempt at closer accuracy was made than to judge whether the sections went one-fourth, one-half, or three-fourths through. The wax duplicate was trimmed accordingly.

2. The necessity of making certain allowances. In general, the theory of wax reconstruction that the straight edges of the wax layers when smoothed down will conform to the sloping edge of the prototype itself and that thus the surface will be equalized evidently applies to such small objects as the individual cells in question. However, it is equally evident that in places it does not. Without going into detail, for the cases where it fails would be obvious to any observer, only two instances will be mentioned. Where a polar section is of the same area as the next adjoining, which frequently occurs, in the nucleus particularly, it is plain that since the outer surface of this polar section is spheroidal, a plate with a straight edge gives substance in excess. Again in the nucleus it occurs not infrequently in polar sections that the boundaries of both its surfaces appear on focusing, one very decidedly smaller than the other, or there may be definite indication of sloping that a straight edge will not account for. In these and other instances where the form of the reconstruction supported the microscopic indication, allowance was made, usually reciprocal, either to supply a deficiency or to remove an excess. However, such modifications were avoided except on very definite indications.

The accuracy, therefore, in regard to these two factors rests upon personal judgment. Most fortunately, however, the success in obtaining sections at one micron lessens their impor-

tance or indeed partially eliminates them. For two micra sections, a fair approximation of duplication depends on judgment and common sense; for one micron sections, it may be made much more mechanical. For the latter, all polar sections were taken as of full thickness and the few allowances that were made changed the results only to the extent of a small fraction. By themselves, all that is accomplished by the two micra series is to eliminate the wider variations in the calculations of individual coefficient figures from the data of simple measurements used in making the averages. By reference to the frequency-polygon (text fig. A) it will be seen that the measurements of single sections of cells taken as they came give coefficients ranging from five to nineteen, though in table 2 the averages of these same cells give fairly constant figures. The ten cells reconstructed from two micra sections narrow the limits of variation practically within one unit and confirm the accuracy of the method of average measurements. The five cells reconstructed from one-micron sections in turn establish finally the essential accuracy of the grosser method of reconstruction and prove beyond question that the ordinarily simpler method of average measurements is sufficiently reliable.

3. The uncertainty of uniformity in cutting the dendrite. The general rule stated for measurements was followed, and usually, with three dimensions to guide, the chance of discrepancy is minimized. In the case of cells whose dendrite bifurcates within a short distance, of which four examples were encountered, it was found that unless the point of bifurcation was included, the results were discrepant (figs. 2, 3 and 4). This confirmed the practice of measuring such cells to include the crotch.

4. The difficulty of seeing the thinnest edge of the section (the maximum cross section of the frustrum it constitutes) to trace it.

5. The indefiniteness of the nuclear margin in the thinnest sections, or, of more weight, the masking of the outline by perinuclear chromatin, which in every resting Purkinje cell is almost certain to be present in a small segment at least.

6. Inequalities in thickness of microtome sections. This is negligible because such series were avoided.

7. The difficulty of cutting straight edges in the thicker wax plates necessitated to correspond to the magnification in the two micra sections. An electrically heated knife was found essential. The cut out wax layer was always checked by superimposing on the tracing and corrected. In several cases, a plate of half thickness was used, and each layer reduplicated.

8. Spherical aberration of microscopic lenses. It was noted with the highest powers that the readings from the object micrometer were appreciably larger toward the periphery of the field. In a cell of ten layers, this would have a decided influence on the weight of the much larger plasma. The higher coefficient figure which resulted on Cell 4, table 2, was thought to be due largely to this, and at least the figure fell without altering consciously any other factor in the repetition. The difficulty in general was obviated by making as many cells as possible from the same plate and at the same magnification.

9. Inequalities in the thickness of the wax plates. This refers to localized inequalities which might occur judging from the appearance of discarded plates. The allowed inequalities within one-tenth of a millimeter probably average up.

10. The limitations in estimating the magnification. The limits of certainty seemed to be within twenty or thirty; beyond that it became an approximation.

3. In the application of the prismoid formulas

1. Incomplete end sections in the series of either the cell or nucleus. Such layers of only a fraction of the full depth could only be computed as cones and added to the results obtained by Simpson's rule.

2. Each cell is divided by the knife into a certain number of layers, each constituting a frustrum, whose bounding parallel planes differ more or less in area according to the slope. The tracing off of these layers from the microscope must in the main follow the outline of the larger area, call it the base of the frustrum. Theoretically, because on one side, for one-half of the spheroidal body, the slope of the edges of the aggregated layers is the reverse

of the other side, one is compelled to substitute on the side of declination of curve the actually estimated area of the basal bounding plane for the other and smaller bounding plane which is the proper one (refer to fig. 5). For the mesial layers, with their lesser curvature of edge, this is negligible, but it becomes of increasing importance with the increase of slope and especially in the last section layer which presumably is conical in shape.

It is plain that considerable variation would be expected in the two micra serials.

4. In fixation and staining

All the material was fixed in formalin-sublimate (95 parts of saturated sublimate to 5 parts of 40 per cent formaldehyde) and stained in erythrosin and toluidin blue. It is believed that the constancy and uniformity of the results will go far in dispelling the bug-a-boo of lack of confidence in technic which has been one of the greatest handicaps to nerve cell study. The fixing fluid was made up as needed, and some of the material had been in paraffin for three years or more. The stain is only a convenient and simple modification of the old eosin and methylene blue combination. In short, it is as much true for the nerve cell as for any cell that the prerequisite is a good fixation, after which any stain proper to that fixation will take. Delafield's hematoxylin stains Nissl substance beautifully when used on properly fixed material ('13 b). After chance for all the contingencies that happen in ordinary laboratory management, the constant relation of cell to nucleus emerges essentially undisturbed. Why should not one expect as much from any other accepted fixative? The actual coefficient figure might or might not be different from that after mercury, it might be the same for the resting cell and differ in the very edematous exhausted cell ('10), but the point is that for that fixative it should be a constant between corresponding cells, and hence perfectly reliable. Yet the tendency has been to lay everything at the door of technic. Variations in size, irregularities of shape, even variations in chromatin are primarily ascribed to fixation and stain. Differences in technic do cause certain alterations

in morphology, mainly of absolute size, but they are very minor and interfere not one whit with the really significant changes of immediate function. After six years of continuous study of a single type of cell, I know of no changes in that cell which have not their explainable origin in function, save only the obvious ones of faulty technic such as lack of proper dehydration, overheated paraffin, and the like. When it is properly done, however done, the essential picture is always constant. Nissl's artefact chromophile cell is present when function has not gone beyond that stage; when function has gone further, Nissl's chromophile cell is absent.

Incidentally, it must be pointed out that conclusions based on cell measurements with the factor of function neglected cannot be exact and are very likely to be incorrect. When one considers that the exhausted Purkinje cell is three or four times the volume of the resting cell and the exhausted crayfish cell even ten or fifteen times the volume of its resting cell, the necessity of limiting comparisons of individuals or averages to corresponding grades of function is obvious. While this restriction is most pertinent to the nerve cell, it must be true in varying degree for all types of functioning cells.

THE BIOLOGIC FACTORS OF DEVIATION FROM A CONSTANT NUCLEUS-PLASMA RELATION

All vital phenomena of cells, as manifestations of transformations of energy, are reducible in terms of three attributes—reproduction, function and nutrition. Growth is not neglected, for, following Hertwig, growth is held to be either divisional or functional. The differentiated nerve cell has lost the power of division. Shifts in the nucleus-plasma relation due to divisional activities are therefore eliminated after the embryonic period. There remain, in a fundamental analysis of cellular phenomena reacting to modify or change the organic structure and relations of the cell, its physico-chemical constitution, just two things, function and food.

As concerns function in the case of the nerve cell, it must first be set forth with what right and upon what basis so vast

a range of functional phenomena in kind and in degree belonging to a cell as a class so complex and variously differentiated, albeit possessing the common physiological attribute of irritability, may be considered as one unit factor. For the range of functional manifestations must include in the matter of degree not only pure activity but the absence of activity—its diametric opposite—and in the matter of kind everything that is not reproductive or nutritive. Fortunately, this right and basis are characterized by an elemental simplicity when the principles are stated. The mere statement will suffice here as the reasoning and the facts upon which it is based have been developed and presented in detail in previous papers.

As to degree of intensity, activity and its diametric opposite, depression, are physiologically analyzed by Verworn ('96) as merely quantitative opposites, "activity being an increase, depression a decrease in the intensity of vital phenomena." Most beautifully complementary to this are the results of anatomical analysis. In terms of the nucleus-plasma relation, activity is an upset of the relation in favor of the cytoplasm. Depression is just the opposite, an upset in favor of the nucleus. In other words, the quantitative opposites in Verworn's physiological sense are just as much quantitative opposites as regards their reciprocal mass relations. However much a condition of life such as heat may change in its range from freezing to overheat, it can and does alter the corresponding reaction only in degree.

As to the kind of phenomena, the common identity manifested in nucleus-plasma correlation and interchange after all possible stimuli, if adequate, means, if it means anything at all, that all excitant stimuli, namely those inducing pure function, produce an identical reaction. The anatomical unity, not merely of superficial and objective homology, but of measured quantity, is invariable. There is the same identity of anatomical reaction after all depressant stimuli, using the word stimulus always in Verworn's broad sense of any change in the environment. Stimuli can only be excitant or depressant, it making no difference that their reaction may combine first excitation, then later depression. It follows therefore that the singleness, the unity of reaction

to stimuli differs only in a quantitative way. The possibilities are only activity or depression. The consideration further therefore as to kind of phenomena becomes merely part of the consideration as to degree, the only essential difference is a quantitative one, and in all respects save degree functional phenomena are identical. In other words, to quote a former conclusion ('13 b) it is the constitution of the nerve cell, for which sensitiveness to stimuli is the specialized function, that, if it reacts at all, whatever the stimulus, it reacts in the natural direction either of exaltation or inhibition of the capacity of a mechanism which serves all phases of function. Function is primarily referable to a quantitative reaction, fundamentally the same in every nerve cell. The facts stated in this paper give the final proof—after all the alternation of increase and decrease of substance for any given activity, hyperchromatism and hypochromatism, the cell comes back to a constant nucleus-plasma relation. It means that there is lacking a specificity or inherent peculiarity in the reaction of one nerve cell as compared with another. Inherent differences in specific cellular powers are not at all demanded in explaining the most diverse function.

This does not exclude qualitative differences of a sort. A difference in chemical quality of protoplasm, specifically of chromatin, is predicated not only phylogenetically but ontogenetically for varying degrees of differentiation ('13 a). The distinction is that as limited within a species this difference in quality is between one individual and another, and within an individual between different types of cells; the quality of the protoplasm within cells of the same type within the same individual is undoubtedly a close constant. Secondly, as a result of natural causes such as repeated cycles of activity producing age or of injurious agencies such as disease, a qualitative deterioration is predicated by such things as a deficiency of elaboration of chromatin and nucleolar substance and a loss and relative slowness of the power of recuperation which is anatomically evident. But the distinction here is that the reaction of a given moment is regarded as starting from the fixed quantitative status belonging to that moment and that reaction so far as concerns the

material elaborated and consumed to consummate it is a quantitative reaction. Thus, for example, in senility, the senile resting cell is an organically damaged cell, shrunken, deficient in substance, perhaps without a karyosome, though withal showing the characteristics of a resting cell and the absence of activity changes. When this damaged cell is excited to activity—and it will react until the nucleus vanishes—it plainly runs a parallel sequence with the most virile young cell in its varied activity changes, and though the substance is less and the nucleus-plasma coefficient changed somewhat, the appearance indicates plainly that the senile changes are quantitatively based on the acquired nucleus-plasma coefficient. So obvious does this appear that it has not been considered necessary to test it by measurements, and, indeed, why should a principle which holds all through life change for a natural end result of that life, especially when the modifications that then take place may be correlated with that principle, as will appear? The decline of quality then with advancing age is conceived to affect rebuilding and recuperation, and so the cell recovers to a lower level of efficiency after each cycle of activity. Each succeeding display of activity is quantitatively based on the last level. Verworn comes to the same conception from the physiological side as the following quotation will show ('09).

Die spezifische Energie irgendeiner gegebenen Form der lebendigen Substanz ist also etwas Relatives, das abhängig ist von dem gerade gegebenen Zustande des betreffenden Systems und nur in bezug auf diesen momentan gegebenen Zustand besteht die Gesetzmässigkeit, dass alle Reize primär die spezifischen Lebensprozesse quantitativ verändern, indem sie dieselben erregen oder lähmen.

Nor does disease offer any exception in its primary analysis. To an extent that has not been sufficiently appreciated, disease is a natural process. Within that process limit, morbid excitant stimuli produce the same changes as normal excitant stimuli and morbid depressant stimuli parallel normal depressant stimuli. Cells are driven to exhaustion in the pure excitation of shock or tetanus; morbid depressant stimuli go no further in their final inhibition of function than a normal condition of life such

as heat in its secondary depression after excitation, when it is carried beyond its normal limits to its final effect ('13b). This is the exact simile because practically all disease stimuli appear to be primary excitants and final depressants. The parallelism would undoubtedly be as true of pure depressants of disease as it is for pure depressants of natural life such as cold or starvation if carried far enough. However induced, depression finally merges into a degeneration—the process ceases and a condition ensues. But most decidedly this does not interfere with the conception that within rather wide limits disease works its effect according to the laws of stimuli as a physiological process, and more than that, after the manner of the process of natural function.

Having thus defined the nature of function, one can proceed to the analysis of the ways in which the factor function may alter or affect the nucleus-plasma relation. Primarily for the present purpose this relates to the effect of function on the resting cell as such, but incidentally its outside effect will enter. Functional activity, function proper, comes first in importance. As sufficiently suggested, it may be analyzed in two groups: First, the purely quantitative reaction of a single process of activity—from rest to exhaustion in a virile animal, as proved by the fact that through the prime of the cell such a process proceeds from and returns to a constant nucleus-plasma relation; second, the alike quantitative reaction in a cell so qualitatively weakened in its power of recuperation as the result of the repeated cycles of activity of a life—from rest to exhaustion, then by recovery back to rest—that it shows organic reduction in its resting state, the senile cell. In the frankly senile cell, the nucleus-plasma relation is changed thereby to a different level.

Concerning the first group of regular quantitative reaction, if the resting cell becomes active, it ceases by that act to be a resting cell. It makes no difference, as I see it, whether that activity be due to excitatory stimulation of whatever sort or whether we admit spontaneous stimulation. Activity, however induced, can only affect the cell in one direction and it must be affected or there would be no activity. It proceeds to a fixed sequence of changes—in order, the enlargement in equilibrium,

the hyperchromatism, the shrinkage with subsiding hyperchromatism, the secondary enlargement coincident with hyperchromatism (functional hypertrophy), etc. The nucleus-plasma changes which result are as fixed and constant as is the relation in the resting cell—they are founded on that relation. Function is not an intangible thing—definite anatomical states subtend it.

The facts already brought out of constant constitution and nucleus-plasma relation prove the resting cell as a type apart. In diverse functional states it has remained a constant. Further, the essential nature of the sequence of changes denominated active denotes that something far and away different in degree is taking place in the functioning cell as distinguished from the resting cell. The changes can only mean a vastly increased intake, upbuilding, coincident with a vastly increased outgo, which latter soon gains the advantage and leads to imbalance of the cell and nucleus. Energy is being rapidly elaborated, rapidly dissipated. Contrast that with the equilibrium of the resting cell which is always the same. It makes no difference how severe or prolonged is the stimulation, how widespread the reaction. Until it is actively engaged, the fixed type of resting cell holds. Even in the most profound activity, it is rare not to find some of this type. And if for illustration only one is left, not only would that one have all the characteristics of stain and content, but the indication appears sufficient that by serial section and reconstruction the ratio of its plasma to its nucleus would be a measured constant. With these facts, one is justified in limiting function to certain numerous types, rest to a single solitary type. With these facts, would it satisfy reason that function passes through an intangible thing, originating from and affecting all types of cells alike? Function cannot affect the resting cell as such without making it a functioning cell. The quantitative reaction of function is eliminated as a biologic factor affecting the nucleus-plasma relation of the resting cell as such.

The second group included in pure function, namely, the quantitative reaction of a single phase of activity which takes

place in a cell qualitatively deteriorated in its power of recuperation after the cycles of activity of a lifetime, whether naturally or prematurely induced, now comes up for discussion. Sufficient has been said to make the above distinction clear—even if the quality of the cell has changed, every immediate reaction must be a quantitative reaction founded on the coincident quality of its resting cell or else the fundamental constitution that makes it a nerve cell has changed. How this change in quality brings about an actual and measurable change in the nucleus-plasma relation may readily be shown. The qualitative deterioration of age harmonically complements in every way the quantitative exhaustion of a single cycle of excessive cell work. In such an immediate exhaustion, the nucleus comes to exhaustion first, and the process of activity ends with the balance of capacity on the side of the plasma ('10). In terms of the nucleus-plasma relation, the relation is upset in favor of the plasma. This is proved by the excessive size of the plasma as stated in terms of numerical coefficients, by the dechromatinization of the nucleus and finally by the marked lagging behind of the nucleus in the process of recovery (Dolley '11 a; Hodge '92). Old age is characterized by an identical result in that the nucleus suffers relatively more. The first observable indication of senility is a progressive irregularity of the nucleus, the 'final fatigue' of Hodge. Next the karyosome goes. The shrinkage continues, the nucleolar substance diminishes, and finally the nucleus disappears, but nevertheless a distinct amount of plasmatic substance remains before complete necrobiosis. In other words, after each cycle of activity the cell comes back to a different level of inferior capacity, the plasma always maintaining the end advantage just as in a single overstrain. In senility, therefore, there is just the same upset of the nucleus-plasma relation in favor of the plasma, which becomes more pronounced at each organic loss of substance and which finally becomes absolute,—there is no longer a trace of nucleus. *A priori*, therefore again, one could most confidently predict that the measured numerical coefficient of this relation would be a higher figure. There has not merely been a loss of recuperable substances, of which chro-

matin is the representative type; there has been a deeper reaching loss of the substances which provide for recuperation, a loss absolutely demonstrated by the final disappearance of the nucleus and later of the plasma. If plasma and nucleus disappeared together, the equilibrium might be expected to have been maintained, that is, there should be no change in the nucleus-plasma relation. On the contrary, the nucleus suffers throughout disproportionately. The inevitable trend, as between the relative size of nucleus and of plasma, is toward a progressively and relatively larger plasma. The present results, so far as the senile resting cell is concerned, bear this out. The figures for the nucleus-plasma coefficients are uniformly higher than any obtained for cells in their prime, that is, their nuclei are relatively smaller (table 5, Senility). Furthermore, as the senile cells measured are limited to those showing shrinkage alone of the nucleus or of the body and nucleus and as the selection stopped at those with so much organic deficiency as loss of the karyosome, the figures prove that this final upset in the relation expresses itself fairly early in the development of senility. This is an important point in the estimation of senile capacity and may indeed be taken as evidence, with the principle established, that the qualitative deterioration of the senile cell has begun. On the other hand, these figures do not differ greatly in size from the usual constant. That is, the changed basis of reciprocal relations, of mutual interchange of materials, is not a marked one, and the cell has not departed greatly from the original basis of equilibrium. Though likely the numerical ratio increases to a steadily increasing degree throughout the senile necrobiosis there is no evidence that the cytoplasm is preserved at the expense of or independent of the nucleus but the invariable picture is that of association of plasmatic decline with nuclear decline, a certain balance is preserved, and so function must continue in the usual quantitative way until the nucleus goes, though on a progressively lower level of capacity.

In explanation of the figures in table 5, it may be said that the first series of Experiment 31 was the first series measured from this animal. Some cells with senile nuclei were included without

TABLE 5
Dimensions, relative volumes and nucleus-plasma coefficients in biological deviations from the resting Purkinje cell norm of the dog

NUMBER OF EXPERIMENT	CHARACTERISTICS OF CELL TYPE	DIAMETERS OF AVERAGE CELL IN MICRA	DIAMETERS OF AVERAGE NUCLEUS IN MICRA	RELATIVE VOLUME OF CELL	RELATIVE VOLUME OF PLASMA	RELATIVE VOLUME OF NUCLEUS	NUCLEUS-PLASMA COEFFICIENT
<i>Senility</i>							
Muscular-exercition 31 (table 1)	{ First series, with senile and normal cells mixed Frankly shrunken senile types Frankly senile nuclei	34.60×19.57	11.94× 8.87	13251	12313	938	13.14
		33.02×16.78	11.09× 7.77	9297	8628	669	12.89
		35.00×18.75	11.70× 8.30	12304	11498	806	14.20
Cerebellar section 4							
<i>Depression</i>							
Normal 18 (an old but not senile animal; weight 15430 grams)	{ First series; normal and depressed resting cells Second series; the same with some cells of Stage 2 Third series; Stage 2 of normal activity in frank depression Fourth series; resting cells in parallel degree of de- pression (Stage 1)	37.88×18.82	12.95× 9.67	13417	12206	1211	10.09
		37.32×20.29	13.58× 9.88	15364	14039	1325	10.59
		39.77×23.17	15.67×11.62	21350	19235	2115	9.09
		34.99×19.43	12.20×10.03	13209	11982	1227	9.76

thought of their making an appreciable difference, since their cell bodies corresponded with the most normal cells (see same animal in table 1). That the higher coefficient figure for this first series was due to senility is proved by the results after selecting cells unaffected by senility (table 1) and corroborated by the second series from Experiment 31 in table 5 of frankly shrunken senile cells. The series from Experiment Cerebellar Section 4 represents also cells with only shrunken nuclei.

It must be specified that the senility here discussed is a natural senility (Dolley '11 a) the final fatigue of Hodge ('94) the increase of plasma of Minot ('90, '08). It does not refer to the other possibility of permanent impairment which would eventually result from a depression carried beyond physiological limits to actual degeneration. That this possibility of depressant senility exists has been conclusively shown by R. Hertwig's studies on the protozoa ('04) in which the depressant upset of the nucleus-plasma relation in favor of the nucleus may become permanent and the animals pass into degeneration and die. From the severer nature of the breakdown of the plasma of the depressed nerve cell, analogous changes may be predicted and will be investigated. Incidentally, it seems clear enough that the views of Minot and Hertwig as to the cause of senescence, "apparently diametrically opposed," as Conklin says in his discussion ('12 a) increase of plasma against increase of nucleus, are each correct and that the difference of opinion arose because there are two inherently opposite factors in senility, of which each investigator considered only one.

So far as concerns function in its inclusive sense, as a factor of disturbance of the nucleus plasma relation, depression, the inhibition of function, remains to be discussed. That depression would change the ratio of nucleus to plasma would be predicated because depression changes the structural relations of every type of cell, whether in rest or in function. The resting cell is as much involved in depression as is any stage of activity in which depression may intervene to inhibit the further progress of that activity. For the full understanding of why that is so as well as to explain the quantitative nature of the changes of depression,

it is necessary to consider depression from the point of view of its metabolism. Activity itself could have been considered from this point of view, since the actual basis for regarding activity and depression as quantitative opposites is a metabolic one. Incidentally, therefore, this discussion will be used to round out the consideration of activity.

The starting-point will be the resting cell, in the strictest sense "a cell in a state of preparedness for work, with any previous waste repaired." There is no external work but "there must be a constant display of internal work in maintaining its structural integrity and carrying on its internal metabolism" ('10). This is the recognized state of "tone." While the next statement in this earlier discussion of nerve cell mechanics was "it is assumed" that there is an exact balance, an equilibrium between cell and nucleus, it is no longer an assumption, for the constancy of the ratio of nucleus to cell both in the same and in different animals of the species proves it beyond a doubt. The assimilative and dissimilative processes of the cell must exactly equal each other. More definitely, what the cytoplasm absorbs of food material, what it gives to the nucleus, what the nucleus returns to the cytoplasm, and finally what is consumed must exactly equal each other.

When now the resting cell is excited to the performance of work, there is an obvious increase of all tangible materials and the cell and nucleus enlarge to a size as much as 50 per cent greater than that of the resting type from which they started. Yet both plasma and nucleus increase in exactly the same proportion, for the fixed norm of the nucleus-plasma relation has invariably held for this first stage of activity. Both the intake and the outgo are increased, likewise the interchange between plasma and nucleus must be increased in both directions, yet the same equilibrium is maintained quantitatively exact. This in itself would seem sufficient to give an idea how function rests upon a quantitative metabolic basis the same in kind but more intense in degree than that of the resting cell. The actual complete identity in substances involved could be shown if the evidence were discussed that the whole metabolic process in both

the resting cell and the working cell is integrated towards the building up of chromatin as at least the principal representative of the end products, if not the end product, whose breaking down furnishes directly the energy of work. For the functioning cell, all the changes that happen after the first stage of proportionate increase represent an attempt to restore the metabolic balance between the same substances which has then become disturbed as a result of the overdrain under continuous stimulation. In terms of chromatin, the consumption of cytoplasmic chromatin (Nissl substance) exacted from the nucleus, comes to exceed the supply available for energy of work, the cytoplasm being the bearer of function; this makes an increased demand on the nucleus; the nucleus, always relatively more affected, is forced to fall back on its reserve and to exact in turn reserve material from the cytoplasm, its only source, and so it undergoes a functional hypertrophy; this reacts on the cytoplasm, thus forced to synthesize more materials for the nucleus and it hypertrophies in turn; the balance is to a degree restored and there results a renewal of the chromatin supply. Finally, when the reserve is exhausted, the cytoplasm exacts the residual chromatin from the karyosome of the nucleus, and the nucleus, unable to contribute to further synthesis, and denuded of its own, is left exhausted. Yet, throughout, the measurable constancy in the shifts in absolute and relative size, each shift interpretable of a purposeful significance in the interchange of material between plasma and nucleus, declares the whole reaction, reserve on reserve, a quantitative one on an exact physico-chemical basis.

Depression, the antithesis of activity, is its diametric quantitative opposite. The genesis of depression is to be found in the breakdown of the plasma in its reciprocal relation to the nucleus. It loses the power to synthesize raw food material as proved by yolk and glycogen deposit, and it loses the power to exact chromatin substances from the nucleus. As a result of the failure of primary synthesis, the nucleus is unable to obtain elaborated materials for its further synthesis, and the nucleolar substance, the plastin, which is ordinarily used up in the formation of chromatin, instead now piles up in the nucleus. Chromatin for-

mation ultimately stops entirely, a thing which becomes objectively very apparent when what was formed previously to absolute depression becomes used up by the cytoplasm. As a result of the progressive loss of power to resorb the chromatin substances from the nucleus, both the residual chromatin of the nucleus and some of the chromatin formed during the advance of depression remain stored within the nucleus, in the latter case a most obvious thing when it takes place in stages which normally have no free chromatin outside the karyosome proper. This bare sketch will serve to indicate that there is a quantitative increase of materials peculiar to the nucleus in depression, marked in profound depression, and quite the opposite of the nuclear exhaustion of activity, nucleolar substance to nucleolar substance, chromatin to chromatin.

A priori, therefore, it would be predicated that depression, obviously a matter of blocking metabolism and consequently affecting the resting cell as much as any functioning cell, would change the nucleus-plasma ratio of the resting cell to make the nucleus relatively larger. However, the eye alone could not determine how great this increase of nuclear materials might be in terms of quantity. It was definite enough that the nucleolar substance was not diminished in depression, and as it was sufficient for the general interpretation that the substance which in activity becomes submerged and vanishes in the synthesis of chromatin should remain at a standstill, it was preferred to err on the side of caution and this remaining at a standstill was emphasized, with the statement of actual increase as a probability ('13 b). Indeed, I rather doubted that anything short of exhaustive measurements would bring out any tangible increase in nuclear materials within the limits of technical variations of measurement.

Fortunately, one of the dogs killed for the purpose of measurement showed a considerable degree of anatomical depression. It was a most unexpected finding and there was no indication of it in his outward behavior so far as a superficial scrutiny went. However, this is by no means the first autogenous depression that has been seen in animals undisturbed by experiment. Not

all the Purkinje cells were affected and the condition ranged from a slight to a marked physiological degree, occasionally to frank degeneration, but more cells were affected than remained normal. The results of measurements are recorded in the second division of table 5. First twenty-five resting cells were measured as usual, taking them as they came whether without depression or with depression of slight, moderate, or marked degree. The coefficient figure, 10.09, was very noteworthy, but still open to doubt. Another set was measured from different sections. The second figure differed only by one-half a point, and was still lower than any variation in the regular series of most likely technical origin. The slightly larger size in the second series is readily explained as due to the purposeful inclusion of some cells just passing from rest into activity and hence slightly larger. As a further test it was decided to measure a series belonging to the initial stage of activity. They are much more favorable because, being toward the maximum of initial enlargement with a fairly uniform hyperchromatism, confusion in diagnosis is impossible and the size is more uniform. Of the twenty-five of this group measured, nine were recorded as slight, nine as moderate, and seven as severe examples of depression. The resultant coefficient was 9.09.

Finally, a fourth series of resting cells was measured. These were most carefully selected, not because they showed a relatively large nucleus but because they exhibited definite standards of depression, and further they were chosen to correspond as closely as might be to the third series in degree of depression, nine being slightly, nine moderately and seven severely depressed. The resulting coefficient figure was 9.76. Granting the accuracy of such selective measurements, the result in the third and fourth series are beyond all reasonable question a result of the disturbed functional state. Depression results in a measurable quantitative upset in favor of the nucleus. The fairly close correspondence of the coefficient figures in the third and fourth series is highly important. The normal resting cell and the first stage of normal activity have a common ratio. So they would appear to have in depression. But in depression this ratio is expressed

by a smaller figure for the resting cell and subsequent events, that is, the amount of activity belonging to a degree of depression, are commensurately based on that lower figure, not on the original norm. In other words, I should be willing to predict that measuring cells of the same grade of depression as it attacks all stages of activity, the changed ratio resulting from that depression would fall below or rise above the changed level of the likewise depressed resting cell for the whole series in proportion to the changed resting cell level, though in the same sequence as for normal activity. This could only happen for a fixed quantitative reaction diminished by a fixed amount of interchange of material. It is a concrete thing that instead of the plasma's being eleven times larger than the nucleus as in the first stage of normal activity, it is only nine times larger in a certain degree of depression for that stage and that these figures belong respectively and identically to the normal resting cell and the corresponding depressed resting cell. This is no more remarkable than the demonstrated fact that as the Purkinje cell progresses from its embryonic state to its nucleus-plasma norm, from a small coefficient figure to a higher one with the growth of the plasma, the changes of activity for any point in that progress are quantitatively based on the figure which subtends the resting cell belonging to that point. Thus, in a ten-day puppy, at which age all phases of activity are definitely recognizable, the nucleus-plasma norm is about four. The changes of activity are definitely based on that figure, rising above or falling below it as happens for the adult for eleven. The undeveloped cell duplicates the mechanism, in Donaldson's apt words ('13) it is a "working model," but the quantitative interchange is less.

Returning to the question mentioned earlier as to whether the nucleolar substance is actually increased in depression, the measurements give now undoubted evidence that it is. It could not be a chromatin increase which is the main factor, because from the nature of the process new formation of chromatin is failing and the continuance of the failing resorption for a time prevents the accumulation in great excess within the nucleus of what may still be formed. Objectively, the excess of intranuclear

chromatin is comparatively small, as may be determined from the resting cell which is normally without chromatin save in its karyosome. In accord with the earlier statement, however, the actual increase of nucleolar substance "only makes more pronounced a phenomenon which is sufficiently significant if that substance maintains a level" and does not affect the interpretation. It is extremely valuable in the evidence it offers that the nucleus has direct metabolic relation with the external medium, as Verworn originally stated ('91) and gives indication that the nucleus synthesizes its own peculiar nucleolar substance from raw materials. In depression, the cytoplasm is primarily affected and the nucleus relatively more spared throughout the process up to final degeneration and so the nucleus continues to elaborate its own nucleolar substance despite the plasmatic breakdown. Howard and Schultz, in their studies on the biology of tumor cells ('11) came to the same opinion of the constant production of nucleolar substance and of its excess in depression:

Whether, in this dependence of chromatin on nucleolar substance, the necessary amounts of the latter are formed only in the nucleus from materials derived from the cytoplasm, or whether nucleolar substance can be preformed as such in the latter situation, cannot be stated definitely. The constant production of nucleolar substance, however, would seem to be a premise required for the explanation of the continuous formation of chromatin and the ceaseless transference of nuclear materials to the cytoplasm. That the production of nucleolar substance may become excessive and exceed all the probable demands of the cell we shall point out later in tumor cells.

Finally, food remains to be discussed as a possible factor of disturbance of the nucleus-plasma relation. The place of food is in constituting the basis of the repair of waste and of the maintenance of the balanced level of reserve capacity in the resting cell, and in supplying the elements for more active and abundant synthesis in the functioning cell. The metabolic play of function rests upon its food. It seems sufficiently established that food alone is not competent to produce an overgrowth (Adami '10). The overgrowth of either plasma or nucleus or both that takes place in the various phases of the nerve cell's activity is solely a functional overgrowth. A proper, even an

excess supply of food, so far as this latter principle is concerned, cannot affect directly the reciprocal relation of plasma to nucleus in the nerve cell. Food is eliminated as a factor on the active side.

Of course, food may affect the relation in the nerve cell indirectly through its known injurious effects on related systems and organs, which may react as either overfunction or depression. But the nerve cell is par excellence a working cell, specialized for that alone, in no sense a storehouse of food, and so it seems likely that it only takes what food it needs according to its state. I have seen nothing in the resting or functioning cell that indicates an excessive piling up of food material, and depression, in which it is such a prominent feature, proves that it could be seen. However, as this must be yet largely theoretical, it must be said to cover the possibilities, that in so far as overnutrition can be a factor, it would lead to depression and eventually to depressant atrophy, as has been proved most conclusively for the Protozoa (Hertwig). So on the passive side overnutrition cannot yet be eliminated as a factor of depression, affecting as such both resting and functioning cells.

The deficiency and the absence of food have an established place. Food is a condition of life, and Verworn has pointed out that the absence of any such factor is primarily potent in producing pure depression. Thus by starvation R. Hertwig and his pupils produced profound depression in the Protozoa and this has been confirmed for the nerve cells of the cat by Mr. T. K. Kruse in this laboratory. However, in harmony with the usual belief that the nervous system is spared at the expense of other organs, the depression produced, even after considerable time, was only moderate in degree. Absence of food, therefore, is only one type of the depression group.

In conclusion, so far as concerns the resting cell as such, it appears established both on a rational and experimental basis that the sole factors which can alter its constant nucleus-plasma norm are two, functional senility and functional depression. For the resting state, the law of species identity applies outside of the working of these two factors.

More broadly, to cover both the resting and the functioning state, the disturbance and possible upset of the nucleus-plasma relation can be produced by the continuance of normal function and by the depression of function. Divisional activities, which are outside this discussion as without application to the nerve cell, constitute the only other primary factor of disturbance of the relation. The disturbance of normal function may be the purely quantitative reaction of a single process of activity from rest to exhaustion in a virile animal. Or it may be the alike quantitative reaction of a single process of activity which starts from a senile resting cell, the distinction being that such a cell is qualitatively deteriorated in its power of recuperation after the repeated cycles of activity of a lifetime, and hence its nucleus-plasma relation has become changed to a correspondingly less efficient level.

Function does not affect the resting cell as such save as an eventual senile qualitative result. The immediate reaction to function is that the resting cell merges into a functioning cell and thus loses its former identity. Depression, since it lowers metabolism in all types whether in function or rest, does affect the resting cell.

THE SIGNIFICANCE OF A CONSTANT NUCLEUS-PLASMA RELATION FOR EVERY CELL OF A TYPE WITHIN A SPECIES

If the deeper significance of the nucleus-plasma relation has been properly interpreted, the obvious working of such a law of a constant quantitative relation common to each particular type of cell of a particular species is to place corresponding cells on an identical physico-chemical basis that I do not believe has been suggested from such evidence from cytological work. Not only is the reaction of a given type of cell an exact quantitative reaction, judging from the measurable and constant changes of activity, but, within rather narrow limits of deviation, there is a common quantitative reaction for this type of cell for the species. What this identity within the species predicates as a part of the nucleus-plasma relation is a closer quantitative unity than the general constitutional unity underlying nerve cells. Phylogenet-

cally and ontogenetically, there is a unity in that the mechanism, the mode of reaction of nerve cells, is fundamentally identical in all animals whatever their differentiation, as declared by the constant trend of the nucleus-plasma curve. Thus the curve from the crayfish duplicates exactly the trend of the curve from the Purkinje cell of man; for every shift in the relation in man, there is a corresponding shift in the crayfish, though somewhat modified. On the side of the stimulus, there is a closer unity in that all adequate stimuli call forth an identical anatomical response. Complementing the whole, this identity within a species of the mass relation of nucleus to plasma comes to unify and standardize even to a numerical constancy the reaction of each type of cell.

A new basis of comparison of the variability between individuals results. Variability comes consequently within narrower limits and is made to rest on fewer factors than is the usual conception. Thus Adami says on the first page of his "Principles of pathology:" "No two living beings, although belonging to the same species and the same family, are structurally identical, nor even born identical; and-if this be true of structure it is true also of the outcome of structure—namely, function. There is thus no absolute standard of either structure or function in any one species." While undoubtedly this is true to a degree which will be clear and of more particular application in the point discussed by him, namely, the difficulty of demarcation between the normal and abnormal, yet it would appear that there is a fixed underlying identity. It is not an identity of entities but of relations of entities. If the uniformity of a given stimulus in kind, duration, and intensity be granted and if the two biological factors of deviation, namely, senility and depression, be excluded, the factors of variability in functioning power and capacity of the nerve cell are reduced to two, first, the absolute differences in size of cells among different individuals, and, second, the most certain differences in the quality of the protoplasm. That the difference in quality of the protoplasm is amply sufficient to explain individual variations when the complicating factors above are excluded the experimental evidence leaves little doubt. The

variations in the collective cellular reaction after the same duration of overstrain, the just as marked variations in the power of recuperation (Dolley '11 a) in which process it may take one animal two weeks to recover to a degree that another reaches in four days, the variations in the viability of nervous tissue as studied in resuscitations after relative death (Crile and Dolley '08) all of these variations, resulting in young animals, depend primarily upon the individual's quality of protoplasm. The mechanism is identical, the substances through which the mechanism accomplishes its work are identical in fundamental constitution, and so there remains only the probability of finer differences in chemical composition ('13 a). Explosiveness is an attribute of gunpowder, but even in ordinary gunpowder there are grades of explosiveness.

With regard to the factor of absolute size of cells, differences therein may be considered according to the more usual distinction at the present time as acquired or innate. As regards acquired differences, functional usage leads to increased capacity, and correlative therewith is the functional hypertrophy of the cell. Though its limits vary greatly, increased size of this sort means increased efficiency for the same cell, and hence, presumably, for a constant quality. This principle is well established. Representatives of the flat type of resting cell vary in their absolute size both in the same and in different individuals as a result of their varying functional hypertrophy. On this account there must be slight variations in intensity of corresponding reactions in even the same individual as well as greater differences between different individuals. This gives acquired absolute size a logical place as a potential factor of variation.

In the case of innate absolute size, it may be said that the predominant phase of the growth of the nerve cell is functional growth, as stated in an earlier section, and that this fundamentally determines absolute size. On this basis, innate growth to an absolute size and a functional hypertrophy, as usually distinguished, have much in common, if, indeed, they do not result from an identical process. Therefore innate absolute size must share a place as a potential factor of variation between indi-

viduals with acquired absolute size and must work the same way, though to what extent is yet to be defined. In fact, from another point of view the inference is necessitated for a purely quantitative reaction that the principle of increased efficiency for increased size must hold, if a constant quality is premised. Other factors being excluded, the absurdity to which this reasoning would lead if there were an exact correspondence of nerve cell size to body size, namely, that the larger animal is necessarily the more efficient animal on the nervous side, cannot follow if the present data are correct. The larger animal has the larger cell only in so far as its peculiar functional demands and functional usage have developed different cells. It may have nerve cells smaller than the mean for its species. Further analysis is impossible until the limitations of innate growth and the relation of function to growth are definitely established for the nerve cell.

This is as far as the facts appear legitimately to carry one. To go further would be pure hypothesis. Yet with this beginning of the recognition of numerically constant quantitative relations, one can hardly doubt that there lie hidden relationships on the phylogenetic side which perhaps will shed further light on the laws governing evolution and on the side of function will lead to the basis of differentiation. Can it be without significance that all the cells of the crayfish are on the same nucleus-plasma level, or does it mean a common degree of differentiation suitable to the crayfish? If it be without significance, why should a spinal ganglion cell have one relation and a Purkinje cell another and so on?

SUMMARY

Numerous facts in the course of previous work pointed to the induction that the individual members of a species are to a remarkable degree on the same level of constancy as regards the quantitative relation of nucleus to plasma for every type of nerve cell. The evidence of the quantitative nature of the nerve cells' reaction with its measured volumetric correspondence and the objective recovery after functional activity to the identical prior level of rest have suggested this induction most directly. Fur-

ther, the development of function in the embryo proceeds in a graded fashion to accord with a constant relation of full development, and finally, the identity of the mechanism in all nerve cells gives the universal application.

The resting type of nerve cell stands apart as the point of departure for function but uninvolved in the immediate effects of function. From its nature, the resting cell affords an exact basis of comparison between different individuals because it may be identified as unaffected by any factors which might complicate the nucleus-plasma relation. Coincidentally with the collateral evidence, several concrete examples of a species constancy were found in the resting Purkinje cell type of the rabbit, dog, and man. As a part of the determination of the relation of the resting cell to the functioning cell in the crayfish, a species constancy likewise eventuated, broadened in this case to include all primal types of resting nerve cells on the same level. Upon the basis of these data, verification to warrant the formulation of a law was sought, as follows:

For the aggregate, calculations were made from the average of measurements of resting Purkinje cells, twenty-five cells to the series, from thirteen individuals of the dog species, which, with those previously made, brings the number of animals to fifteen. The material includes a most heterogeneous assortment of animals of widely varying weights, of all ages, of both sexes, of mixed and fairly pure breed, of different grades of nutrition, and, finally and most important, of a wide range of both natural and experimental functional states. The single condition of ineligibility imposed was the existence of frank functional depression which may affect the resting cell as such.

For the individual cell, calculations were made from the data from serial sections at two micra and at one micron in two ways. First, wax models were reconstructed and the relative weight of the wax plasma to the wax nucleus was obtained on the theory that the quantitative relations of their prototypes would be preserved. Second, application was made mathematically of the prismoid formulas. Fifteen cells were reconstructed in wax, ten after two micra serials and five after one micron serials,

from two animals used in the aggregate computations, one an undisturbed normal, the other after a condition of surgical shock. Eleven cells, seven at two micra and four at one micron, belonged to the shocked animal, and four cells, three at two micra and one at one micron belonged to the normal. The prismoid formulas were applied to all one micron cells and to three two micra cells.

As tested by all these methods, the nucleus-plasma coefficient holds to a close numerical constancy for all members of the dog species investigated. The actual dispersions of the ratios of nucleus to plasma amid the individual differences of the animals and particularly as set against their wide variations in size are relatively small and appear to be negligible because they permit of explanation on inherently technical factors alone. After three methods, the results fall in the close neighborhood of a common figure, namely, about eleven: the differences between the arithmetic means belonging to each of the methods are such as were predicted by mathematicians beforehand from the nature of the data; and dispersions from the mean become reduced to the minimum in the more mechanical and hence more reliable results after one-micron serials. For the one-micron cells, the arithmetic mean is 11.129, the standard deviation $\pm .2037$, the coefficient of variation .01830, and the extreme dispersions from the mean $+.272$ and $-.325$.

CONCLUSIONS

1. The law is thus formulated: Resting undepressed nerve cells of corresponding type of all individuals of a species have a mass relation of nucleus to plasma which is a close numerical constant, whatever the age between full development of the relation and senescence, whatever the size of the animal, irrespective of variations in the dimensions and absolute size of the cells both in the same animals and in different animals, and irrespective of the degree of function in excited cells of the same type in the same animal.

The nucleus-plasma relation theory of Richard Hertwig is a law for the nerve cell.

2. The resting nerve cell as a type distinct from the numerous phases of the functioning cell is morphologically fully defined. In addition to its general characteristics, it has the nucleus-plasma relation common to its type for the species.

3. The sole biologic factors which change the constancy of the nucleus-plasma norm of the resting cell as such are two, functional depression and functional senility.

4. Under this law, the principle of the quantitative nature of the nerve cell's reaction is established. Correlative with the evidence for an exact quantitative interchange between plasma and nucleus for every phase of functional activity, the reaction of function starts from a fixed quantitative ratio of nucleus to plasma and returns in recovery to that same ratio under normal conditions until old age incapacitates.

5. A new basis of comparison of the variability between individuals results from a fixed identity of mass relations for a species. On the side of the cellular reaction, if the special biologic factors of deviation, namely, functional senility and functional depression, be excluded, the factors of variability in functioning power and capacity between one individual and another are reduced to two—first, the differences in absolute size of cells, and second, the differences in the quality of the protoplasm.

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PLATE 1

EXPLANATION OF FIGURES

Figures photographed at natural size, reduced to one-half in reproduction.

1 Cell 11 (table 2) at one micron. Thickness wax plate 2.56 mm., magnification 2560 by Zeiss comp. oc. 18, homo. oil imm. 2 mm., tube length 140, stage level. Number of layers for cell body 23, for nucleus 11. Area of layers in s.cm: 2.6; 5.5; 7.7; 10.1; 11.5; 16.7—1.1 (nucleus begins); 18.7—1.6; 21.5—1.6; 23.1—3.2; 18.3—3.5; 19.6—4.1; 16.4—2.6; 16.3—2.0; 16.8—1.9; 15.0—1.5; 14.5—1.2 (nucleus ends); 13.5; 12.3; 9.3; 9.3; 7.2; 5.3; 2.9. In this cell the surface was smoothed down after weighing. Note the atypical shape with a circular indentation.

2 Cell 16, at one micron. From the same plate and magnification. Number of layers for cell body 22, for nucleus 11. Area of layers in s. cm: 4.0; 9.0; 13.1; 16.4; 22.6; 28.9; 28.4; 28.8—0.9 (nucleus begins); 31.3—2.8; 29.2—3.3; 28.6—4.5; 27.6—5.1; 25.2—5.8; 24.0—5.0; 23.4—3.9; 21.0—2.7; 18.0;—2.1; 16.4—0.9 (nucleus ends); 15.2; 11.6; 9.3; 7.1. The cell was evidently sectioned obliquely. The axone comes off from the side. As regards function, it represents a transition to the first stage, and hence is considerably larger than its two regular mates in table 2.

3 Cell 12, at two micra. Thickness of wax plate 2.182, each layer being reduplicated, magnification 2180 by Zeiss comp. oc. 12, homo. oil imm. 2 mm., tube length 166. Number of layers for cell body 7, for nucleus 3, making a very flattened cell in depth. Opened to show nuclear insertion.

4 Cell 13, at two micra. From the same plate and magnification. Number of layers for cell body $11 + \frac{1}{2}$, for nucleus $5 + \frac{1}{2}$.

5 Cell 14, at two micra. Thickness of wax plate 5.189, magnification per micron 2595 by Zeiss comp. oc. 18, homo. oil imm. 2 mm., tube length 141. Number of layers for cell body $9 + \frac{1}{2}$, for nucleus $3 + \frac{1}{2}$.



A NOTE ON THE DEGENERATION OF THE FASCICULUS CEREBRO-SPINALIS IN THE ALBINO RAT

S. WALTER RANSON

From the Anatomical Laboratory of the Northwestern University Medical School

ONE FIGURE

The cerebro-spinal or pyramidal tracts of the rat decussate in the medulla and run through the spinal cord in the ventral part of the posterior funiculi (Spitzka '86, Von Lenhossék '89, Bechterew '90, Goldstein '04, Van der Vloort '06 and King '10). More recently it has been shown that the pyramidal tract is not well medullated in the adult rat and that it consists of fine and medium-sized medullated fibers and great numbers of non-medullated axons (Ranson '13). Because of its incomplete medullation it stains a light grayish blue in Pal-Weigert preparations, while in pyridine-silver preparations the closely packed darkly stained axons give the tract a dark brown color which clearly differentiates it from the remainder of the substantia alba.

The degeneration of this tract after destruction of the motor cortex has been studied in the rat with the Marchi stain by Goldstein, Van der Vloort and King. These studies have shown that the medullated fibers in this tract are of cortical origin. In my previous paper I assumed that the non-medullated fibers of the tract have the same origin, namely, from cells of the motor cortex. No evidence was presented to show that the motor cortex was actually the source of these fibers, and there remained open the possibility that the non-medullated fibers in the pyramidal tract were not pyramidal fibers at all but a separate tract arising from lower centers of the brain, joining the pyramidal tract, decussating with it and accompanying it along its course through the cord. While this possibility is indeed remote, it seems worth while to present definite proof of the cortical origin of the non-medullated fibers of the pyramidal tract.

In nine adult white rats the skull was removed from over the dorso-lateral surface of the anterior half of the left cerebral hemisphere and the underlying cortex was burned away. The attempt was made to destroy all of the cortex on the upper and lateral surfaces of the anterior half of the hemisphere without injuring the underlying structure.

After periods varying from 45 to 60 days, the rats were killed. In each case the entire head was preserved in formalin. The seventh cervical, eighth thoracic, and fifth lumbar segments of the spinal cord were removed and prepared either by the Pal-Weigert or the pyridine-silver technique. Four of the rats were given a preliminary injection of ammoniated alcohol, as suggested by Huber, before the cords were dissected out and the chosen segments subjected to the pyridine-silver technique. This preliminary injection of ammoniated alcohol through the arteries decreases the shrinkage which results from fixing the tissue in strong alcohol. The details of the method are given by Ranson ('12) and Huber and Guild ('13).

After the head was thoroughly hardened in formalin the brain was dissected out and the extent of the lesion on the surface of the brain carefully determined and recorded in notes and drawings. The left hemisphere was then cut into four or five sagittal sections and these were examined under the binocular dissecting microscope to determine the depth of the lesion.

The extent of the lesion varied somewhat. But in each case the larger part of the upper surface of the anterior half of the left hemisphere had been burned. In one case, No. 7, the lesion was small and did not reach the mid-line, and in this case the sections of the cord show that a considerable part of the motor cortex had escaped injury. In the others with somewhat larger lesions practically all of the motor cortex was destroyed, judging from the completeness of the degeneration of the pyramidal tract in the cord.

In each case, except No. 7, the lesion extended to the lateral ventricle which usually appeared as a thin-walled cyst through which could be seen the corpus striatum and sometimes the cornu ammonis and thalamus. So far as could be determined by a

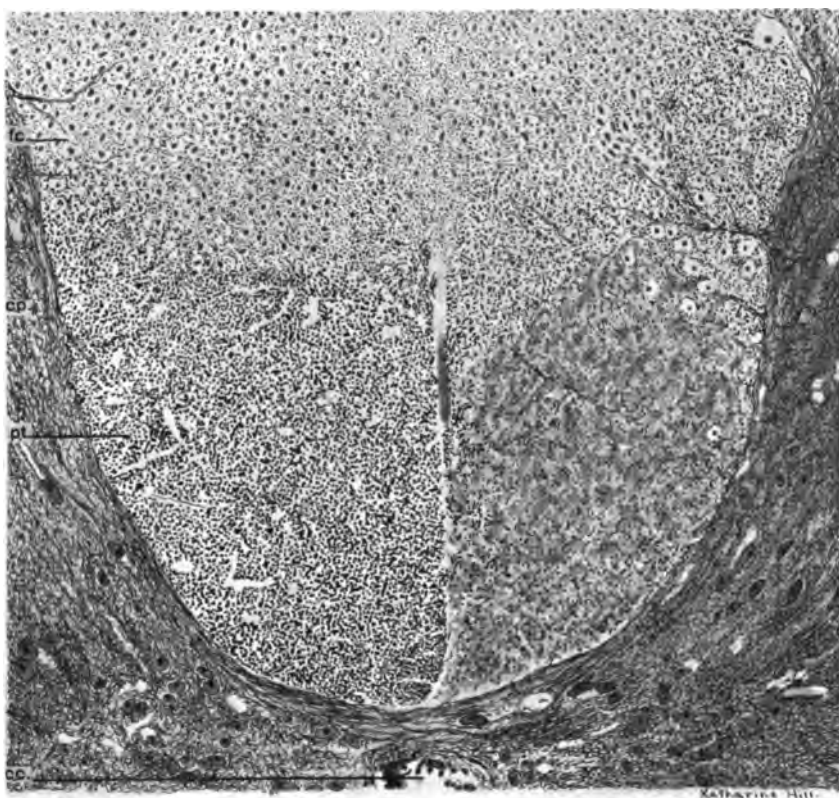


Fig. 1 Ventral part of the posterior funiculus of the spinal cord of a rat in which the motor cortex of the left hemisphere had been destroyed 50 days before the animal was killed. The pyramidal tract of the right side has degenerated. Pyridine-silver. $\times 167$.

study of free hand sections of the hemisphere under the dissecting microscope, no damage had been done to these underlying structures; although there is a possibility that the highest part of the corpus striatum may have been slightly damaged in some cases.

The spinal cords of rats Nos. 1, 2, 3, 4, 6 and 7 were prepared by the pyridine-silver method and all except that of No. 7 showed an almost complete degeneration of the pyramidal tract on the right side. The contrast between the normal and degenerated

side is sharper than could be obtained by the Marchi stain, since, as Miss King has shown, few of the medullated fibers are sufficiently large and well medullated to give a good Marchi stain. In the silver preparations the degenerated pyramidal fibers have disappeared and the somewhat shrunken area of the tract is occupied by neuroglia which stains a light yellow in sharp contrast to the normal tract which is packed with deeply stained fine axons. Scattered through the degenerated area are a few normal fibers. These either represent pyramidal fibers from portions of the motor cortex that escaped injury or an admixture of endogenous fibers in the pyramidal tract. Some of these normal fibers were seen in the degenerated area in all of the cords.

The contrast between the normal and degenerated tracts was not nearly so sharp in Pal-Weigert preparations. For this there are three reasons. In the normal tract the myelin sheaths are very thin and inconspicuous; and the entire area much lighter than the surrounding white substance. There was a considerable number of normal-looking medullated fibers in the degenerated tract. These must either have come from uninjured parts of the motor cortex or have been of endogenous origin. Furthermore, the period of 45 to 60 days did not prove sufficient for the complete absorption of the degenerated myelin sheaths, the remains of the degenerated myelin showing in Pal-Weigert preparations as tiny blue spots. Because of the normal faint staining of the tract and the survival in the degenerated tract of a considerable number of normal medullated fibers, the contrast between the normal and operated sides would not be pronounced even after all the degenerated myelin had been absorbed. As it is, with the remains of the degenerated myelin sheaths showing as tiny blue spots the degenerated tract is scarcely lighter than normal and it requires the use of the higher power lenses to determine which side is degenerated.

It may be that in the minds of some who have read the accounts of the results obtained with the pyridine-silver method on the spinal cord there may have been a suspicion that neuroglia fibers were being confused with nerve fibers. Although there is an abundance of other reasons for ruling out the possibility of

confusing neuroglia fibers with non-medullated nerve fibers, it still may be worth while to call attention to this additional evidence of the nervous character of this group of non-medullated fibers, namely, that they degenerate in the cord after destruction of their cells of origin in the cerebral cortex. These experiments also demonstrate that the non-medullated fibers contained in the area occupied by the pyramidal tract in the cord are true pyramidal fibers, since they come from the motor cortex.

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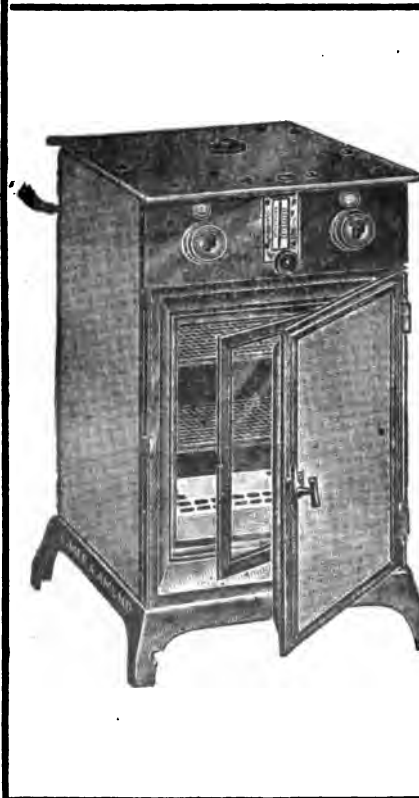


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THE COMPARATIVE ANATOMY OF THE PYRAMIDAL TRACT

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From the Anatomical Laboratory of the Northwestern University Medical School

EIGHT FIGURES

The pyramidal tract, fasciculus cerebro-spinalis or fasciculus cortico-spinalis, has furnished a very interesting topic for investigation on account of the variations in location of this tract in the spinal cord. There are also interesting variations in the size of the tract and in the size of its fibers. Of special interest is the late medullation of the tract in man, and the failure of medullation to complete itself in certain animals, as the rat (Ranson) and mole (Draeseke).

The principal object of this investigation was to determine the degree of medullation in adult animals of various orders. The work was undertaken at the suggestion of Prof. S. Walter Ranson and carried out under his direction.

The animals used were those which were readily obtained and yet represented, as far as possible, different orders of the Mammalia. The Rodentia were represented by the rat, rabbit and guinea-pig; the Insectivora by the ground-mole; Carnivora by the cat and the Primates by the monkey.

REVIEW OF THE LITERATURE

It has been repeatedly shown that the fasciculus cortico-spinalis or the pyramidal tract in the Mammalia may lie in any of the funiculi of the cord, that its size diminishes as it proceeds caudalward, and that it often disappears upon reaching the lumbar region. In the cords of the mouse, rat and guinea-pig, this tract is found in the posterior funiculus of the cord; that in the rabbit and in the Carnivora (Spitzka) is seen to traverse the lateral funiculus; while in man it is located, in part, in the lateral and in part, in the anterior funiculus (v. Lenhossék '89).

Simpson ('12), whose observations were made on the raccoon (*Procyon lotor* Linn) noticed that most of the fibers in the tract after decussating in the posterior part of the medulla, passed into the lateral funiculus of the cord. These were accompanied by a few fibers which were uncrossed and came from the pyramid of the same side. A fair number of fibers from each pyramid ran caudalward, without decussating, in the ventral or anterior funiculus not unlike that in man. From this he concluded that the direct pyramidal tract which was thought limited to man and the anthropoid apes, is present in the raccoon but it disappears about the middle of the thoracic region. This location of the pyramidal tract fibers differs from that seen in the cord of the Canadian porcupine (*Erethizon dorsatus* Linn). Here he (Simpson '12, '13) employed the degeneration method and then stained the sections according to the Marchi technique. After the decussation of the pyramids, most of the fibers crossed to the opposite side and upon reaching the posterior funiculus occupied the anterior or ventral portions of the fasciculi cuneatus and gracilis. Few of the crossed fibers entered the lateral funiculus. A great number of fibers arising from the pyramids do not cross but are continued into the cord in the anterior funiculus along the margin of the anterior median fissure. These form a comparatively large bundle which is compact. A very scanty number of uncrossed fibers were also seen to lie in the posterior funiculus of the same side. Hence in this animal, the fibers of the pyramidal tract are divided into four fasciculi upon entering the cord, viz.: a direct anterior pyramidal tract; a direct posterior pyramidal tract; a crossed posterior pyramidal tract, and a crossed lateral pyramidal tract. Most of the fibers are found in the crossed posterior tract; then next in number are those in the anterior (direct) tract; next in the lateral crossed tract, and finally in the direct posterior tract. Similar results were obtained by Mellus ('99) who confined his work to the motor paths in the monkey's cord. He found that after he had divided the tract at the decussation of the pyramids, the vast majority of degenerated fibers crossed to the lateral funiculus of the opposite side while the remainder passed to the lateral funiculus of the same side.

Also some fibers were clearly seen in the anterior funiculus along the anterior median fissure and these traversed the cervical and thoracic regions where they disappeared.

Rothmann ('10) employed the degeneration method in his study of the pyramidal tract and corroborated the statement that the lateral cerebro-spinal fasciculus in man as well as in the higher mammals, contained crossed and uncrossed fibers but stated that no anterior or direct pyramidal tract was present in the latter animals. Probst ('99) in his work with cats and dogs also employed the degeneration method and obtained results which differed from those of the above investigators. He found that in eleven instances he could only make sure of a suggestion of an anterior pyramidal tract, while in two cases he could see a definitely marked anterior pyramidal tract. Thus he maintains that, in most instances, several fibers of an anterior pyramidal tract can be seen, while in many instances, cats and dogs have an anterior pyramidal tract which is analogous to that found in man. Both of these writers employed the Marchi stain in preparing their sections.

In the case of marsupials, Ziehen ('97) who employed the Pal-Weigert stain in his work, found that the majority of the fibers of the pyramidal tract after decussating, were located in the lateral funiculus, yet a small number wended their way into the anterior funiculus.

The striking resemblance of the pyramidal tract of a rat to that of a squirrel was shown by Goldstein ('04). In both these animals the crossed fibers ran caudalwards in the posterior column occupying its most anterior part. As seen in his figures, the tracts were quite definitely outlined when the Marchi stain was used.

The varied conclusions reached by the different investigators who used the Marchi method in staining their material may be due, as stated by Rothmann ('10), to the Niederschläge or precipitates. These little specks, Körner, which can be met in the field and which often form groups should not be interpreted as degenerated fibers when the Marchi method is resorted to in the study of the tract. This same difficulty was encountered

by Bischoff ('00). No such precipitation occurs when the pyridine-silver technique is used as outlined by Ranson ('12) and as described in this paper, and the results are uniform, i.e., as far as the staining of the material is concerned.

The size of the fasciculus cortico-spinalis or pyramidal tract varies with the different species. Schäfer claims that it is directly proportional to the number and complexity of the movements which an animal is capable of executing (King '11). Thus the pyramidal tract of sheep as found by that investigator (King) is comparatively small and is composed of very fine fibers. Likewise Spitzka ('86) asserts that "the intellectual rank, or what is the anatomical index of such rank: preponderance of the highest nerve centers is not the only factor in determining the size of the pyramids. The dimension of the animal has undoubtedly some influence." The tract of a rat whose extremities are used very much, is found to be better developed (Bectterew '90). He also says that this tract is no less or perhaps better developed in cats and dogs, while in man and the primates the development is at its height. The tract diminishes in size, however, as it extends caudalwards. The fibers diminish rapidly in number (King '11) and the tract disappears in many species before reaching the lumbar region. To give a relative idea of the size of the pyramidal tract in various species, v. Lenhossék ('89) selected a certain same level of the cords in the different animals and then letting the transverse area of this level represent 100 per cent, he expressed the area of the tract in terms of it. Thus at the level of the middle of the cervical cord, the pyramidal tract of the guinea pig occupied three per cent of the transverse area of the cord; in the rabbit, 5.3 per cent; and in the cat, 7.76 per cent. This would tend to prove the statement made above about the factors governing the size of the tract.

All of these investigators used the Weigert, Pal-Weigert, Marchi, hematoxylin-eosin and other common stains and were able to study only the medullated fibers. Not until the pyridine-silver stain was utilized could the composite structure of the pyramidal tract be noted. Thus in his paper on the pyramidal tract in the albino rat, Ranson ('13) has shown that in addition

to many fine and medium-sized fibers which have a medullary sheath, there are a goodly number of non-medullated fibers. From this he concluded that in the case of the rat, the medullation of the fasciculus cortico-spinalis was incomplete. Scattered sparsely throughout the entire white substance of the rat's cord were seen non-medullated fibers but those in the pyramidal tract were very numerous and compact. There is a great difference in the size of the medullated fibers of this tract in different animals. In the rat the small size of the fibers seems to be associated with the fact that large numbers are non-medullated. Do the proportion of medullated fibers and the size which the largest medullated fibers have attained, go hand-in-hand as indices of the stage of development that the tract has reached? With this problem in mind it seemed of interest to determine the size of the largest of these fibers in the pyramidal tract and the relationship between the medullated and the non-medullated fibers in this tract in different mammals.

TECHNIQUE

The arbitrary level of the seventh cervical segment was taken and all sections were made at this region. In most cases, more than two animals of each species were used, since poor preparations were rejected. For each species cords were selected which were of about the same size. Some of the material was stained according to the pyridine-silver method (Ranson '12), the steps of which are as follows:

The tissue is denuded of its dural sheath and placed into

- (1) Absolute alcohol plus 1 per cent strong ammonia for forty-eight hours
- (2) Distilled water, wash for two minutes
- (3) Pyridine, for twenty-four hours
- (4) Distilled water, for twenty-four hours with many changes of the water
- (5) Silver nitrate solution 2 per cent (2%), for three days, kept in the dark and at 35°C.
- (6) Distilled water, rinsed
- (7) Pyrogalllic acid 4 per cent (4%) in 5 per cent (5%) formalin, for one or two days
- (8) Dehydrate in alcohol
- (9) Clear in xylol
- (10) Imbed in paraffin
- (11) Cut sections 10 microns thick, mount and examine

To bring out the myelin sheaths in the pyramidal tract, similar pieces of cord were stained according to the Pal-Weigert technique after fixing the denuded cord in Müller's fluid for one month. The cut sections were from 12 to 22 microns in thickness, and only those were utilized which were at the level of the junction of the seventh cervical nerve roots with the cord. The exact level of the sections was not determined in the case of the mole. The entire cervical cord of one mole was prepared by the pyridine-silver technique and cut into serial sections. Preparations were also made from the thoracic and lumbar segments.

OBSERVATIONS AND RESULTS

Pyridine-silver preparations showed the pyramidal tracts of the rat, guinea-pig and mole so clearly differentiated that no question arose as to their topography. The non-medullated fibers are stained very dark, giving the tract a dark brown appearance which differentiates it from the rest of the white substance. In these sections the contrast is greater than could be obtained by the Marchi method, for in animals such as these the enormous numbers of non-medullated fibers give the tract a very characteristic appearance in the pyridine-silver preparations.

In the sections of the spinal cord of the rabbit, cat and monkey where the contrast is not very sharp, the results of other investigators, who used the embryological and the degenerative methods, were used to locate the tracts. In all cases the identification of the tracts is based on the results of the older methods. The pyridine-silver stain shows that the area which other investigations have shown to be occupied by the pyramidal tract is more or less sharply marked off from the rest of the white substance because of its large content of non-medullated fibers. Only in those cases where, as in the rat and mole, these greatly predominate, would the pyridine-silver method be of service in tracing the bundle downward from the brain.

Rat

The cortico-spinal tract of the albino-rat is situated in the posterior funiculus in the form of two compact bundles separated from each other by the posterior median septum and bounded anteriorly and laterally by the gray substance of the cord, while posteriorly it extends to about one-fourth of the thickness of the posterior funiculus (Ziehen '99, Bechterew '90, Goldstein '04, Van der Vlort '06, and Ranson '13).

As seen in figure 1, from pyridine-silver preparations, the tract is quite clearly outlined; its fibers not intermingling, to any extent, with others in the posterior funiculus. The axons are closely packed and very numerous. Most of them are very small and these are stained dark brown or black. The larger axons are stained yellow while the myelin sheaths are colorless and the neuroglia is stained very faintly or not at all. The preponderance of the very small axons gives the tract a characteristic brown color.

The Pal-Weigert preparations showed the tract as a light grayish-blue area in the posterior funiculus. It contains numerous very fine medullated fibers and a few of medium size. The myelin sheaths are not equally stained and some are very prominent while others are barely visible. They are not as closely packed as the axons in the pyridine-silver preparations. The majority of the axons are non-medullated.

The medullated fibers vary markedly as to their size (diameter). The largest ones as found in these sections, measured 8.44 microns, while the majority of these fibers ranged from 1 micron to 3.2 microns.

Guinea-pig

The pyramidal tract of the guinea-pig is also found to be situated in the posterior funiculus. It differs from that found in the rat in that it is not a compact uniform mass but the two tracts have the form, more or less, of the letter 'V' and the posterior boundary is indefinite. It occupies the most anterior part of this funiculus and its 'horns' occupy fully two-thirds of the thickness of the posterior funiculus. The posterior septum separates the tracts.

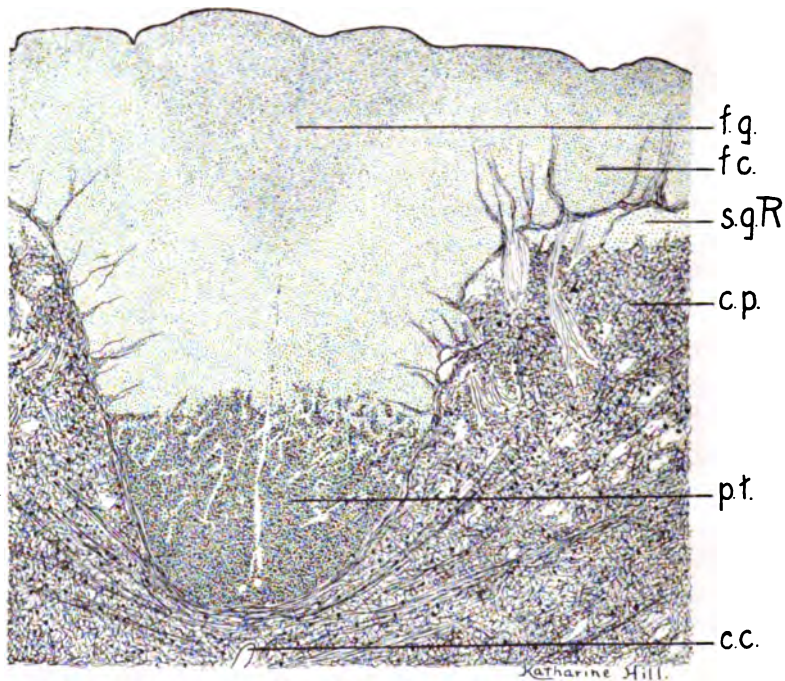


Fig. 1 From the seventh cervical segment of the spinal cord of the albino rat. Pyridine-silver. $\times 93$. The fasciculus gracilis stains somewhat darker than the fasciculus cuneatus because it contains chiefly medium-sized fibers, while the fasciculus cuneatus contains many very large ones. Large medullated axons stain light yellow and their myelin sheaths are colorless, hence those regions in which they predominate stain lightly by the pyridine-silver method. The fasciculus cerebro-spinalis or pyramidal tract is located in the ventral part of the posterior funiculus and is very deeply stained because it is composed of deeply staining non-medullated and fine medullated fibers closely packed together. Nerve cells stain deeply and are indicated in the drawing in solid black.

ABBREVIATIONS

<i>L.t.</i> , Lissauer's tract	<i>c.a.</i> , columna anterior
<i>f.c.</i> , fasciculus cuneatus	<i>c.a.a.</i> , commissura anterior alba
<i>f.g.</i> , fasciculus gracilis	<i>f.m.a.</i> , fissura mediana anterior
<i>e.r.z.</i> , entering root zone	<i>c.c.</i> , canalis centralis
<i>f.c.s.</i> , fasciculus cerebello-spinalis	<i>d.f.</i> , decussating fibers of pyramidal tract
<i>p.t.</i> , fasciculus cerebro-spinalis—pyramidal tract	<i>s.g.R.</i> , substantia gelatinosa Rolandii
<i>c.p.</i> , columna posterior	<i>r.p.</i> , radix posterior

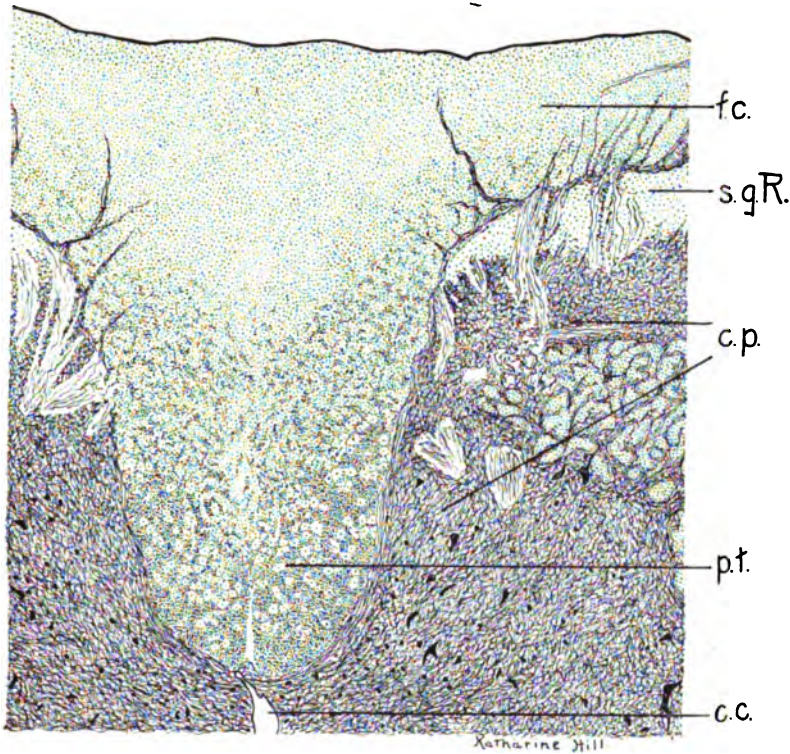


Fig. 2 From the seventh cervical segment of the spinal cord of the guinea-pig. Pyridine-silver stain. $\times 80$. The pyramidal tract is located in the ventral part of the posterior funiculus. It is darkly stained because it is composed chiefly of non-medullated and fine medullated fibers. It is not as compact as in the rat, since there are scattered through it large medullated fibers belonging to the cuneate fasciculus. This is especially evident in the posterior part of the tract, where it appears as bundles of fine fibers in the ventral part of the cuneate fasciculus. The fibers of the pyramidal tract are more densely grouped ventrally and laterally near the gray substance and this gives the cross section of the two tracts somewhat the form of the letter V.

As seen in figure 2, the tract is composed of irregular groups of axons which are closely grouped near the posterior horns and the transverse portion of the gray substance, while medially and posteriorly they are more widely separated. The axons were not as numerous as in the rat but were of about the same di-

ameters. This reduced number accounts for the lighter staining quality of the tract, which however was prominent enough to leave no doubt as to its topography.

The Pal-Weigert sections at this same level show the area occupied by the tract to be of light blue color while the rest of the substance has a somewhat deeper color. The outline of the tract is very indistinct. The myelin sheaths are larger than those seen in the rat cord and their number is quite small as compared with the number of axons seen in the pyridine-silver preparation.

On tracing the tracts caudalwards the pyridine-silver preparations show the tracts to have about the same shape at the level of the fourth thoracic segment. At the level of the eighth thoracic segment the tracts assume a crescent shape, and a very diffuse, indistinct and very markedly decrease in size. There is an evident decrease in the number of axons. When the level of the twelfth thoracic segment is reached, the tracts, though quite distinct, are smaller and consist of two compact groups of axons which have become separated at the posterior median septum. Some groups of axons are seen scattered throughout the posterior funiculus. Proceeding caudalwards the tracts become less distinct and at the level of the second lumbar segment the groups tend to move posteriorly and separate from each other. They have no definite outline and stain very lightly. From here on, the tracts narrow markedly and fade in color and at the level of the fifth lumbar segment they consist of two narrow strips on each side of the posterior median septum. They take a very light stain, the fibers are markedly reduced in number and the tract is barely visible.

v. Lenhossék ('89) and Bechterew ('90) located the tract in the posterior columns and mention the fact that it is not a compact mass as in the rat, though it is more developed than in the mouse. v. Lenhossék also expresses the area occupied by the tract at the level of the middle cervical segment, as being 3 per cent of the total transverse area of the cord. Rebeley and Simpson ('09), who also located the tract in this region, state that the fibers diminish rapidly in number as they travel caudalwards.

The medullated fibers were found to be of greater diameter than those found in the rat, and the largest measured 12.58μ in diameter. The largest fibers in the guinea-pig cord probably are admixtures from the fasciculus cuneatus. Most of the medullated fibers measure from 1.39μ to 4.84μ in diameter.

Rabbit

The spinal cord of the rabbit does not stain well by the pyridine silver method. The results are improved by a preliminary injection of 2 per cent ammonia in 95 per cent alcohol (Huber¹). This fixed the cord 'in situ' and increased the sharpness of the staining. The area occupied by the pyramidal tract is indicated in figure 3. It is located in the lateral funiculus close to the

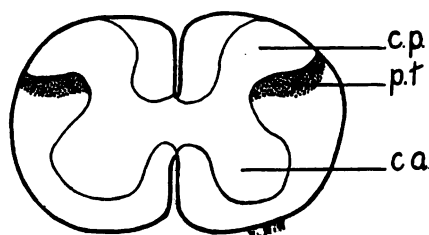


Fig. 3 Diagram of the cervical cord of the rabbit according to v. Lenhossék, showing the position of the pyramidal tract.

columna posterior and near the periphery of the cord (v. Lenhossék). The tract is not sharply outlined in pyridine-silver preparations but the area which it occupies is somewhat more darkly stained than the rest of the white substance.

v. Lenhossék ('89) says that the tracts occupy, approximately, the posterior third of the lateral funiculus in the upper cervical region and at the level of the middle cervical part, the tract occupies 5.3 per cent of the cross-section area of the cord. In the words of this investigator, the tract is located as follows:

Im cervical Teil erkennt man Folgendes: Das marklose Gebiet nimmt den hintersten Teil der Seitenstränge ein. Hinten grenzt es

¹ Observations on the peripheral distribution of the nervus terminalis in Mammalia. *Anat. Rec.*, vol. 7, p. 253, 1913.

unmittelbar an die Rolandosche Formation, nach innen an die graue Substanz, mit welcher es, vielleicht nur weil die Elemente der Grenzschicht noch marklos sind, zu einem gemeinsamen farblosen Felde verschmilzt. Seitwärts reicht es in seiner hinteren Hälfte bis zur Peripherie, in seiner vorderen wird es jedoch nach außen von der um diese Zeit schon myelin haltigen Kleinhirnseitenstrangbahn umsäumt.

Munzier and Wiener ('02) also located the pyramidal tract in the lateral funiculus only. Ziehen, who employed the Marchi stain, found degenerated fibers in the anterior and posterior funiculi besides those seen in the lateral funiculus.

In Pal-Weigert preparations the area occupied by the pyramidal tract cannot be recognized. The lateral funiculus stains equally throughout. In the pyridine-silver preparations it is obvious that the non-medullated fibers are very numerous, but they do not stain as sharply as in the cords of other animals.

The medullated fibers are very large in diameter and the largest found measured 17.44μ , while the majority of these fibers measured 11.3μ to 15.6μ in diameter.

Cat

The relative size of the pyramidal tract as compared with the transverse area of the cord in the cat is greater than in the animals already examined. Its location corresponds to that found in the rabbit. It has a somewhat irregular outline, is very large and is situated in the posterior half of the lateral funiculus. It is in close proximity with the posterior horn and extends to about 2 mm. from the periphery of the cord.

This corresponds with the findings of v. Lenhossék ('89) who localized the tract by the degeneration method. He also states that this tract occupies 7.76 per cent of the transverse area of the cord at the middle of the cervical cord. Probst ('99), on the other hand, who confined his experiments to dogs and cats, states that in eleven animals he could locate a faint anterior pyramidal tract.

The pyridine-silver preparations at the level of the third cervical segment show the tract stained somewhat deeper than the rest of the white substance though its outline is not sharp. Lower down at the seventh cervical segment and as seen in figure 4,

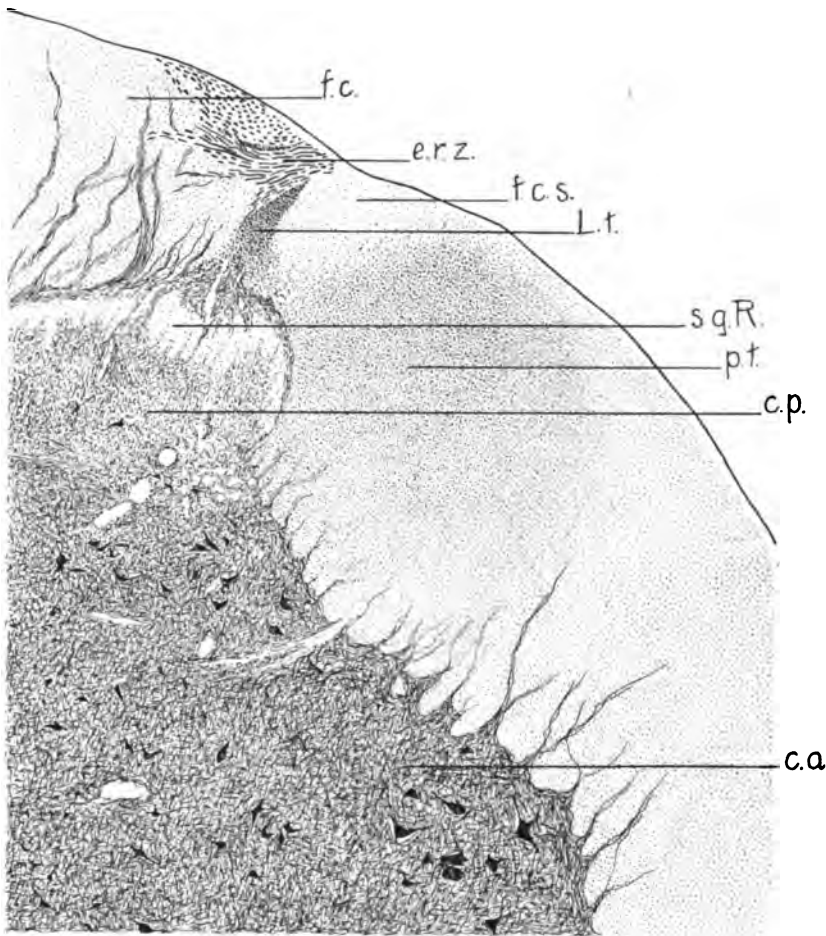


Fig. 4 From the seventh cervical segment of the spinal cord of the cat. Pyridine-silver. $\times 40$. Of all the tracts the cerebello-spinal fasciculus stains the lightest. It is composed almost exclusively of large medullated fibers whose axons are light yellow and whose myelin sheaths are colorless. The fasciculus cuneatus, which is composed of large and medium sized medullated fibers with very few non-medullated axons, stains somewhat darker than the preceding tract. The pyramidal tract, containing small and medium sized medullated fibers and many non-medullated axons, stains still more darkly. Its outline is sharply indicated posteriorly where it borders upon the cerebello-spinal fasciculus. Ventrally and laterally its outline is less distinct. The tract of Lissauer stains much darker than any of the others and is composed of scattered fine medullated fibers and great numbers of non-medullated axons. These differences in the structure of the various fasciculi of the cord have been discussed in detail by Ranson ('13, '14).

which represents the postero-lateral quadrant of the cord, its posterior and lateral borders are quite apparent. In the thoracic and lumbar segments the tract is stained a little deeper than the rest of the white substance of the cord but its outline is not very sharp. In the lumbar region the tract does not extend so far laterally but appears to hug the posterior horn. Its exact outline can not be made out. In the sacral cord the tract is more indistinct and scarcely perceptible.

Examination of the Pal-Weigert sections failed to reveal any indication of the outline of the tract. The white substance of the cord stained equally throughout and the myelin sheaths appeared equally distributed. The axons in the pyridine-silver preparations greatly outnumber the myelin sheaths seen in the Pal-Weigert sections.

The largest of the medullated fibers were equal, in diameter, to those in the rabbit cord and measured 17.44μ . The majority of these fibers, however, measured from 9.67μ to 12.27μ in diameter.

Ground-mole (Scalopus aquaticus)

The presence of a pyramidal tract located in the anterior funiculus of the cord of the ground-mole was discovered by Draeseke ('04). He speaks of it as being an oval gray field situated in the anterior funiculus on either side of the anterior median septum which divides it. His sections were prepared by the old Weigert method after being hardened in Müller's fluid, formalin and alcohol. To him the tract appeared like the gray substance about the central canal and he says that few medullated axons are present. In his study of the tract he also utilized the different modifications of the Weigert stain as well as the hematoxylin-eosin stain and could readily trace the tract through the thoracic region. Following the tract toward the brain he found no decussation of the pyramids.

In the sections of the mole's cord stained by the Pal-Weigert method, examined in the course of this work, a gray oval tract was found in the anterior column just about midway between the transverse part of the gray substance and the anterior pe-

riphery of the cord. The anterior median fissure traversed the field.

As Draeseke ('04) has pointed out and as seen in figure 5, the tract is nearer the gray substance than the ventral border of the cord. The myelin sheaths are very small in diameter and are sparsely scattered in the field. It is due to marked reduction,

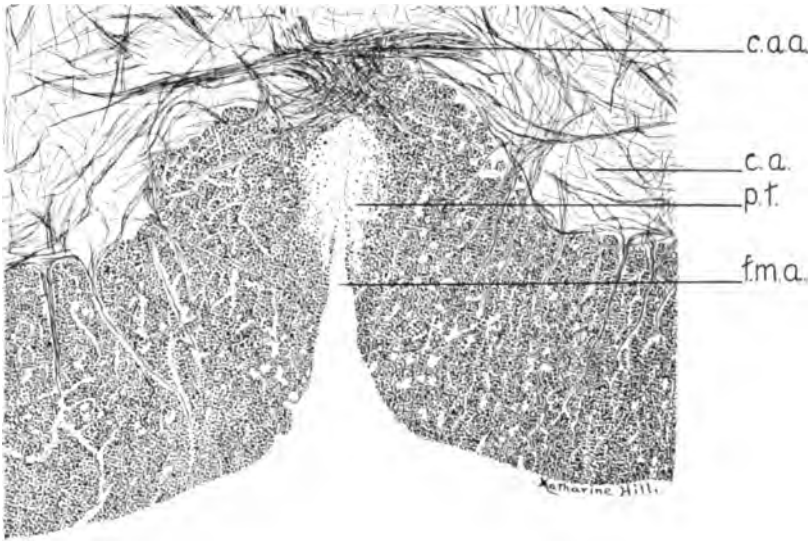


Fig. 5 From a section taken somewhat below the middle of the cervical portion of the spinal cord of the mole (*Scalopus aquaticus*). Pal-Weigert. $\times 93$. The pyramidal tract is located in the anterior funiculus and forms a sharply outlined unstained area. The few medullated fibers which it contains are probably admixtures from other fasciculi of the ventral funiculus.

or one might say, almost total absence of these sheaths, that the tracts remain colorless in Pal-Weigert preparations. In measuring the diameter of these sheaths the largest found in the field measured 4.84μ and are probably admixtures of fibers from the other tracts in the ventral funiculus. The rest of the white substance of the cord was stained light blue due to the presence of numerous myelin sheaths and had the appearance, typical of the white substance in other cords.

In the pyridine-silver preparations the tract is seen to be prominently set off from the rest of the white substance (fig. 6). It is stained deep brown. The axons are densely packed in the field and very small. It is due to their great numbers as compared with the myelin sheaths, that the tract stains so heavily

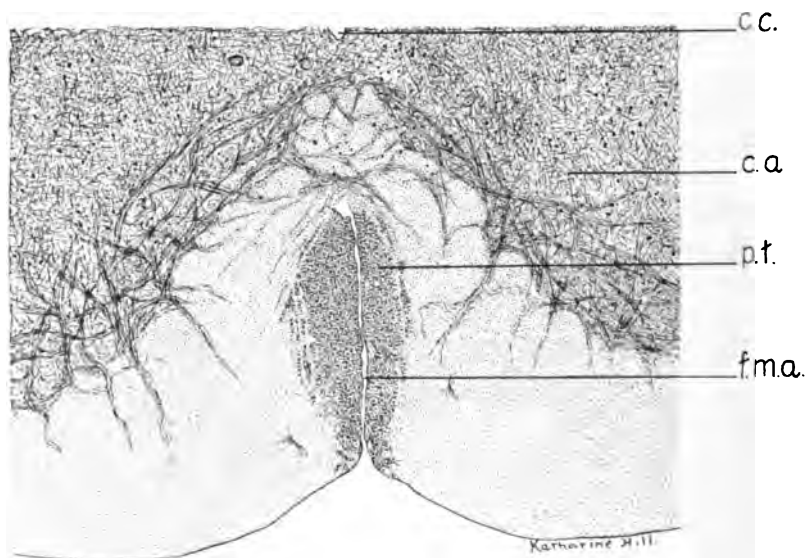


Fig. 6 From a section taken at about the first cervical segment of the spinal cord of the mole (*Scalopus aquaticus*). Pyridine-silver. $\times 93$. The pyramidal tract is larger than in figure 5, is deeply stained, and sharply outlined from the rest of the ventral funiculus. This deep staining is due to the closely packed fine non-medullated fibers of which it is composed.

and stands out so clearly from the rest of the white substance which has a very light brown color. In tracing the tract caudalwards through a set of serial sections of the entire cervical cord, it was found that the anterior median fissure bisected this oval field only at certain levels; while at others, some axons extend across the fissure thus connecting the two tracts by a bridge of decussating fibers. Such a bridge across the anterior median fissure would extend through about 13 consecutive sections of 11μ thickness. In different sections the same bridge of fibers would occupy a different antero-posterior level. At times it would

interrupt this fissure near its anterior end, then it would bisect the fissure, as seen in figure 7, and finally it would be seen near the anterior commissure, the septum extending almost through the entire field. This process would repeat itself after an interval of two or three consecutive sections in which no axons were seen

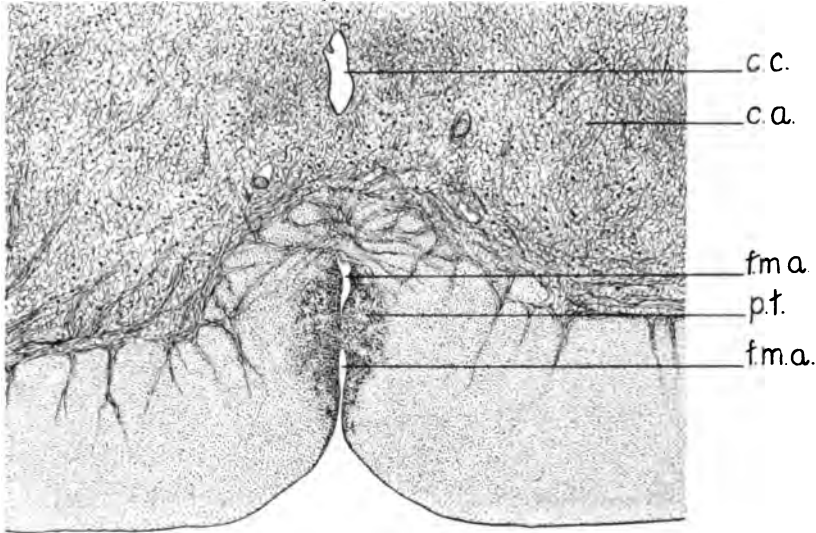


Fig. 7 From a section taken somewhat below the middle of the cervical portion of the spinal cord of the mole (*Scalopus aquaticus*). Pyridine silver. $\times 93$. Compare with figure 6 and note the rapid decrease in the size of the tract as it is traced caudalward. A bridge of decussating fibers unites the two tracts across the anterior median fissure. The marked flattening of the anterior surface of the cord is a distortion produced when the cord was removed.

in the path of the anterior median fissure. This succession of bands of decussating fibers extending across the anterior median fissure and separated by open intervals was found to be coextensive with the pyramidal tract and to cease at the lower level of the cervical cord with the termination of the pyramidal tract. These observations seem to indicate a decussation of the pyramidal tract in the cervical portion of the spinal cord.

A study of serial sections of the entire cervical cord of the mole shows that the size of the tract decreases rapidly as it runs

caudalward. This is seen on comparing figure 6 from about the first cervical segment with figure 7 from somewhat below the middle of the cervical cord. In traversing this short distance it has lost more than half of its fibers. It disappears in the lowest cervical or highest thoracic segments.

The location of the pyramidal tract in the mole corresponds with that found in the hedgehog by Bischoff ('00). He employed the degeneration method and stained his sections by the Marchi method. The degenerated fibers could be traced, through the brain, from the cortex into the upper cervical segments but beyond that the tract could not be followed. The pyramidal fibers were very fine and had a very delicate myelin sheath. They did not cross at any point during their course but continued on the same side of the cord in the anterior funiculus.

Monkey (Macacus rhesus)

In the spinal cord of the monkey (*Macacus rhesus*) the pyramidal tract is located in the lateral funiculus. This fasciculus is elliptical in outline, quite large as compared with the transverse area of the cord, and is situated in the posterior half of the lateral funiculus bordering medially on the posterior horn, while laterally it extends to within 3 or 4 mm. from the periphery. It is not so sharply differentiated in pyridine-silver preparations, as that of the rat or guinea-pig or mole, yet in figure 8, which represents the posterior and lateral quadrant of the cord, its outline is clearly seen.

Mellus ('99) in his work on the brain and cord of the monkey found that the direct pyramidal tract does exist in some monkeys and that it is quite possible for it to vary in individual animals. He also states that the difference in the size of the fibers depends upon the portion of the cortex with which they connect. He arrived at this conclusion by employing the degeneration method which showed that degeneration of the largest fibers followed lesions in the highest portions of the brain near the top of the central fissure or the zone governing the movements of the large toe. Not all of these fibers were large, for many smaller ones

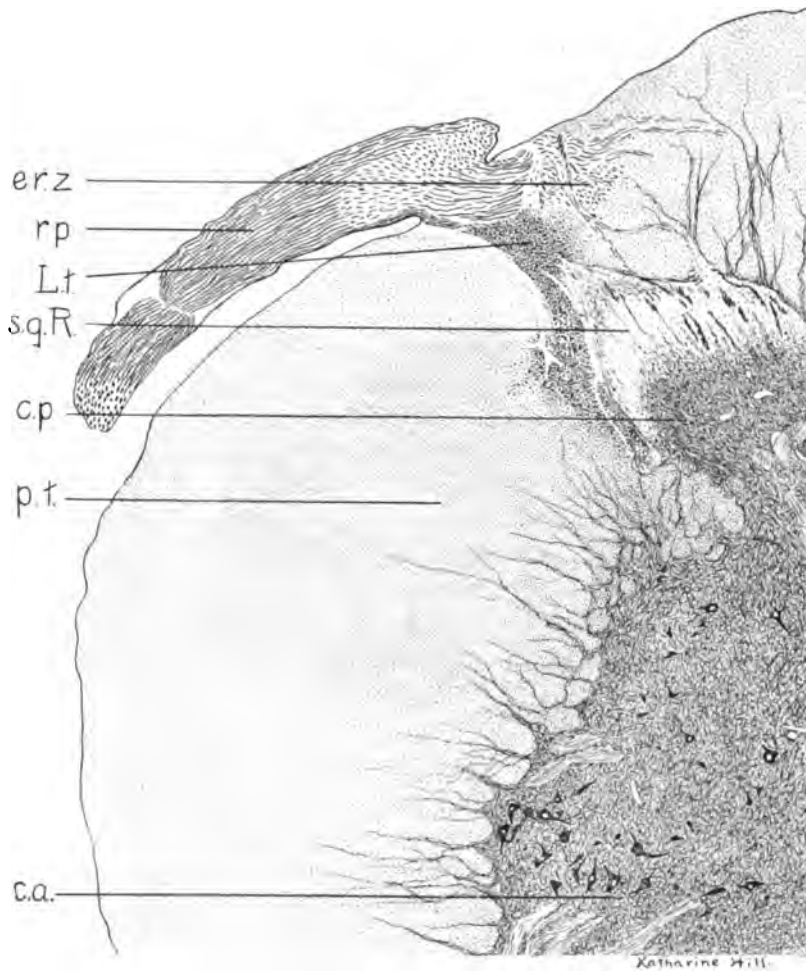


Fig. 8 From the seventh cervical segment of the spinal cord of the monkey (*Macacus rhesus*). Pyridine silver. $\times 40$. The pyramidal tract is a rounded, somewhat darkly stained area near the columna posterior in the lateral funiculus. It is sharply limited posteriorly but its ventral and lateral boundaries are less distinct. The variations in the intensity of the staining of the different fasciculi are the same as in the cat's cord and are dependent upon the same fundamental differences in their component fibers.

TABLE 1

ANIMAL	DIAMETER OF LARGEST MEDULLATED FIBERS	DIAMETERS OF MAJOR- ITY OF MEDULLATED FIBERS
Rat.....	8.39 μ	1.0 μ - 3.23 μ
Guinea-pig.....	12.58 μ	1.39 μ - 4.84 μ
Rabbit.....	17.44 μ	11.3 μ -15.6 μ
Cat.....	17.44 μ	9.67 μ -12.27 μ
Mole.....	4.84 μ	
Monkey.....	11.95 μ	4.8 μ - 9.6 μ

were intermingled with these, but the average size of the fibers appeared to diminish in proportion to the distance of the lesion downward on the convexity of the brain.

Rothmann ('10) who experimented with apes (*Macacus rhesus*) found that the pyramidal tract was situated exclusively in the posterior part of the lateral funiculus. He also employed the degeneration method and could see no degenerated fibers in any other part of the cord and so concluded that no direct pyramidal tract was present. He summarized his paper by saying that the numerous experiments on apes, dogs and cats permit the statement that no direct pyramidal tract in the anterior funiculus is present in the higher mammals, man excepted.

The sections at the level of the third cervical segment show the pyramidal tract not very deeply stained with silver. At the level of the seventh cervical segment and as seen in figure 8, the tract is somewhat more deeply stained and its outline is quite distinct. The axons stand out clearly but their number is small as compared with that found in a similar area of the pyramidal tract of the rat. The medullated fibers are, however, much larger in diameter, and but few small ones are found throughout the tract. In the thoracic part of the cord, the tract is stained a little deeper than the rest of the white substance from which it is not sharply marked off. Lower down in the cord, no suggestion of its presence was shown by the pyridine-silver stain.

The Pal-Weigert sections showed no difference in the staining quality of the white substance and the outline of the tract could not be made out at any level of the cord. The myelin sheaths

present in the area which the tract occupies, were of different diameters and closely packed. Even in the monkey the non-medullated fibers of the tract outnumber those which are medullated.

The average size of the medullated fibers was less than in the cords of the cat or rabbit, but greater than in the cords of the rat and guinea-pig. The largest found measured 11.95μ in diameter, while the majority of these fibers measured from 4.8μ to 9.6μ in diameter (table 1).

SUMMARY

1. The pyramidal tract of the higher mammals contains both medullated and non-medullated fibers.

2. In the mole the tract is almost completely non-medullated. In the rat it is very imperfectly medullated.

3. The tracts decussate in the medulla oblongata in all animals except the mole, where a decussation is suggested throughout the course of the tract in the cervical part of the cord.

4. The tracts may lie in any funiculus of the cord. In the mouse, rat and guinea-pig this tract lies in the posterior funiculus; in the rabbit and the carnivora, in the lateral funiculus; and in the ground-mole, in the anterior funiculus.

5. They traverse the entire cord in all animals studied except in the mole where they terminate with the cervical cord.

6. The imperfect medullation of the pyramidal tract in these animals is of interest in connection with its late medullation in man. The degree of medullation in man can not be studied, since no satisfactory preparations are available.

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AN EXPERIMENTAL STUDY OF LISSAUER'S TRACT AND THE DORSAL ROOTS

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FIVE FIGURES

As a dorsal root enters the cord it divides into two parts. The larger medial portion consists of medullated fibers and enters the cuneate fasciculus; the smaller lateral portion consists of a few fine medullated fibers with great numbers of non-medullated fibers and enters Lissauer's tract. These and the following introductory statements are made on the basis of our studies on the tract of Lissauer, published in 1913 and 1914. In these papers the related literature has been fully considered.

The tract of Lissauer is composed of small, somewhat widely separated, medullated fibers, and a great number of fine non-medullated axons. The number of medullated fibers entering the tract from the dorsal root is not sufficient to account for all the medullated fibers found there. For this and other reasons it seemed certain that the majority of the medullated fibers in the tract were not derived from the dorsal root but were endogenous. Since a much greater number of non-medullated fibers enter the tract from the dorsal root, it seemed fair to assume that a majority of these fibers in the tract came from this source. It was shown, however, that some of these non-medullated fibers were of endogenous origin, at least in certain animals. In the rhesus monkey, for instance, the tract spreads out into the lateral funiculus along the side of the columna posterior. Since no oblique fibers were found running from the dorsal roots towards this lateral expansion of the tract, the fibers, both medullated and non-medullated, which were found in this part of the tract, were considered to be of endogenous origin. It seemed probable

that a part of the non-medullated fibers in the tract of the cat and other animals in which this lateral expansion is not so well developed, were also of endogenous origin.

The present investigation was begun with a three-fold object:

1. To determine what proportion of each of the two kinds of fibers in the tract are of endogenous origin.

2. To trace by the method of degeneration the course in the cord of the non-medullated fibers of the dorsal roots, and to find where these fibers terminate.

3. To determine whether or not any of the non-medullated fibers of the dorsal roots are efferent, i.e., arise from cells in the spinal cord.

TECHNIQUE

In this investigation cats were used because the normal structure of the dorsal roots and tract of Lissauer has been most carefully studied in this animal. Furthermore, the spinal cord of the cat was known to react in a uniform and favorable manner to the pyridine-silver stain which was to be employed in preparing the material.

Nine adult cats and one half-grown kitten were used, besides those in which for various reasons the operation was unsuccessful. A laminectomy was performed and the dura exposed over the sixth and seventh lumbar and first sacral roots (under the sixth and seventh lumbar spinous processes). In the first three operations the exposed roots (both ventral and dorsal on one side) were ligated proximally to the ganglion without opening the dura. Strong braided silk was tied very tightly about the roots in such a way as to insure a complete degeneration. In the other seven the roots (both ventral and dorsal on one side in the exposed area) were cut proximally to the ganglion. In these the dural sheath was of necessity opened by cutting through its prolongations on the roots.

An interesting phenomenon was the rapid development of shock following the division of the roots. An animal would remain in good condition until this stage of the operation was reached. Immediately after the roots were cut, the animal would show signs

of collapse and die of shock in from ten to thirty minutes. It was found that one five-hundredth of a grain of atropin given hypodermatically a few minutes before the roots were cut, while not entirely eliminating shock, prevented its fatal termination.

TABLE 1
List of experiments

NUMBER	ROOTS	OPERATION	DURATION	STAIN
I	L 6, 7, S 1	ligated	24 days	pyridine-silver
II	L 4, 5, 6	ligated	24 days	pyridine-silver
III	L 6, 7	ligated	74 days	Pal-Weigert
IV	L 5, 6, 7	cut	74 days	pyridine-silver
V	L 6, 7	cut	24 days	Marchi
VI	L 7, S 1	cut	70 days	pyridine-silver
VII	L 7, S 1	cut	54 days	Pal-Weigert
VIII	L 6, 7	cut	20 days	Marchi
IX	L 6, 7	cut	14 days	Marchi
X	L 6, 7	cut	51 days	pyridine-silver

At the autopsy the roots were identified and it was determined positively which roots had been injured. Each of the lumbar and the first two of the sacral segments of the cord were identified and the cord was then divided into these nine segments which were kept separate for histological study. These segments were so large that they required further subdivision to secure the penetration of the fixing fluids. So far as possible, serial sections were made of these subdivisions of the segments. Since the material from Cat III was to be fixed in Müller's fluid, the segments did not require subdivision, and the entire sixth lumbar segment was cut into serial sections. In Cats IV and VI the segments were split longitudinally into ventral and dorsal halves, of which only the dorsal halves were preserved and prepared by the pyridine-silver method without further sub-division. This rendered possible the cutting of serial sections of the entire dorsal halves of the segments immediately involved in the operation.

The Pal-Weigert and Marchi methods are well known and require no explanation. The technique of the pyridine-silver method has been given elsewhere (Ranson '12).

DEGENERATIVE CHANGES FOLLOWING DIVISION OF THE
DORSAL ROOTS

Twenty-four days after the operation the dorsal roots were in an advanced stage of degeneration. Fragmentation of the axons was complete and most of the resulting debris had been absorbed. The greater part of the degenerated myelin had also disappeared. The degenerated fibers were being transformed into nucleated protoplasmic bands. This was the condition of the roots up to the point at which they entered the cord, or, more exactly, up to the point at which the connective tissue of the root gave place to neuroglia. Levi ('06) has shown that in the lumbo-sacral region this occurs just outside the cord. At this point in the root just outside the cord there was an abrupt change in the stage which the degeneration had reached. In the entering root and in the cuneate fasciculus the degeneration had not progressed so far as in the extra-spinal part of the root, and large irregular fragments of axons were seen in big globules of degenerated myelin. The difference was due no doubt to the presence of sheath cells on the fibers outside the cord which materially aided in the disintegration and resorption of the fibers.

Almost all of the fibers in the root as it enters the cord were degenerated. There were, however, in the degenerated roots of Cats I, II, IV, VI and X a varying small number of fine axons of normal appearance, which entered the cord along the same paths as the original non-medullated fibers. Also in Cats III and VII a few fine medullated fibers of normal appearance were seen entering the cord from the degenerated roots. These fibers may either have been efferent dorsal fibers, whose cell bodies were within the cord, or they may have been regenerated fibers growing from the root into the cord. For reasons which will be given in another paragraph I am inclined to regard them as regenerated fibers; nevertheless, these experiments do not enable us to exclude the possibility of the occurrence of efferent fibers in the dorsal roots. But the experiments do show that at least the vast majority of the non-medullated fibers degenerate in the proximal part of a divided dorsal root. This would indicate that their cells of ori-

gin are situated distally, that is, in the spinal ganglion. These experiments confirm in this respect the results obtained by a histological study of the dorsal roots and their ganglia (Ranson '12).

There are no points of interest in connection with the degeneration in the posterior funiculus. These phenomena have been so often described that it would be a waste of space to discuss them here. Figure 1 shows the degeneration which had occurred in the sixth lumbar segment 74 days after the sixth and seventh lumbar roots had been tied off on one side. Figure 3 shows the degeneration which had occurred in the seventh lumbar segment 20 days after cutting the sixth and seventh lumbar roots.

Although the degeneration in the posterior funiculus has been repeatedly described in great detail, I have not found in the literature a satisfactory account of the changes in Lissauer's tract following lesions of the dorsal roots. The recorded observations may be summarized as follows: Nageotte ('03) reported a case in which a tumor involved all the nerve roots in the cauda equina up to and including the fourth lumbar, without causing any degeneration of the medullated fibers of Lissauer's tract. Many human cords, in which extensive degeneration of the dorsal roots had resulted from tumors, syphilis, and other causes, as well as the cords of animals in which some of the dorsal roots had been divided, have been studied with the object of tracing the degenerating fibers within the spinal cord. Most of these investigations fail to demonstrate any changes in the tract of Lissauer, which has, in fact, received very scant consideration (Darkschewitsch '96; Fröhlich '04; Kopeczynski '06; Marguliés '96; Orr '06; Wallenberg '98; and Zappert '98). A small amount of degeneration in Lissauer's tract after lesions of the dorsal roots has been seen by Collier and Buzzard ('03), Laignel-Lavastine ('08), Sibelius ('05) and Sottas ('93).

As shown in figure 3, degeneration of the dorsal roots was accompanied in our experiments by a degeneration of medullated fibers in the medial half of Lissauer's tract. In this region degenerated medullated fibers were clearly evident in Marchi prepara-

tions 20 days after division of the corresponding nerve roots. They are limited quite sharply to the medial half of the tract, the lateral half being free from degenerated fibers.

In Pal-Weigert preparations at the level of the injured roots one sees that the medial part of Lissauer's tract is more lightly stained and contains fewer fibers on the operated side than on the normal side, while there seems to be no change in the number of fibers in the lateral part of the tract (figs. 1 and 2). Figure 2 represents a part of the tract on the injured side of the cord; the right side of the drawing is from the medial half of the tract and the left side from the lateral half. It will be seen that there is a marked reduction in the number of medullated fibers in the medial as compared to the lateral half of the tract. Normally the medial and lateral halves of the tract have about the same number of medullated fibers.

As to the length of the dorsal root fibers within Lissauer's tract, the best information is given by Marchi preparations from Cat VIII, in which the sixth and seventh lumbar roots were cut. In the sixth and seventh lumbar segments there is a well-defined degeneration in the medial half of Lissauer's tract as seen in figure 3. In the fifth lumbar segment, the first segment above with intact roots, there is a much smaller number of degenerated fibers and these are present only in the medial half. In the fourth lumbar segment, the second above with intact roots, there are a very few degenerated fibers in the medial half of the tract. In the third lumbar segment there are none. It thus appears that some of the medullated fibers run upward one segment, and a very few as much as two segments. In a downward direction the medullated fibers do not extend so far. In the upper part of the first sacral segment, the first below with intact roots, there are a few degenerated fibers in the medial half of Lissauer's tract; but in the lower part of this segment there are very few indeed. If a section through the lowest part of the seventh lumbar segment is compared with a section through the highest part of the sixth lumbar segment (Cat VIII, L, 6 and 7 roots cut), it is seen that the number of degenerated medullated fibers which extend

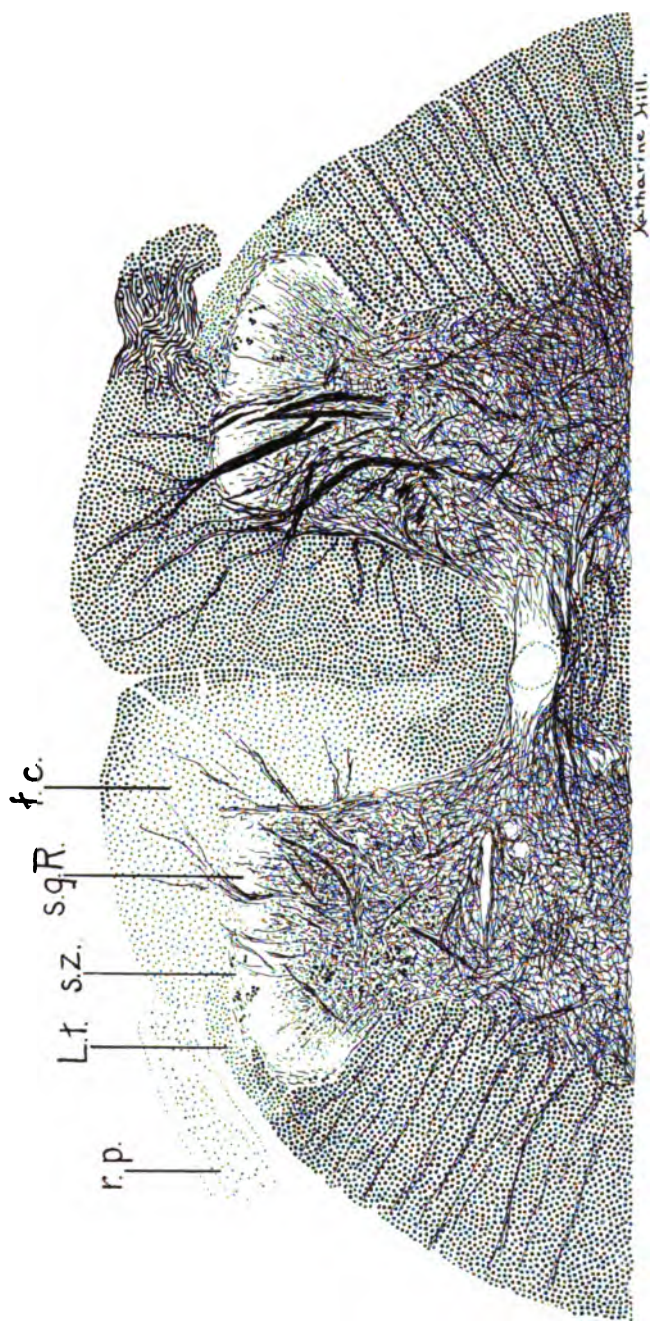
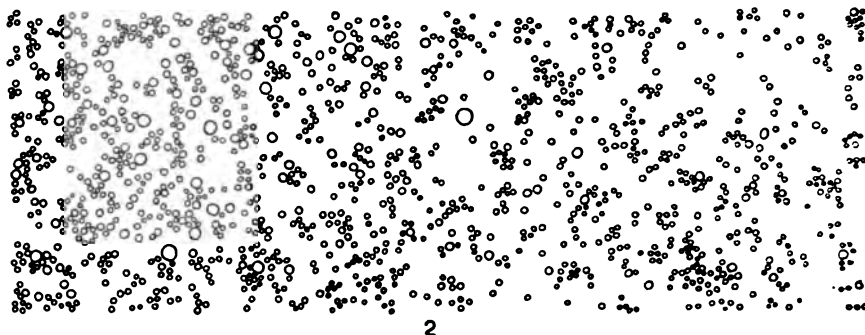
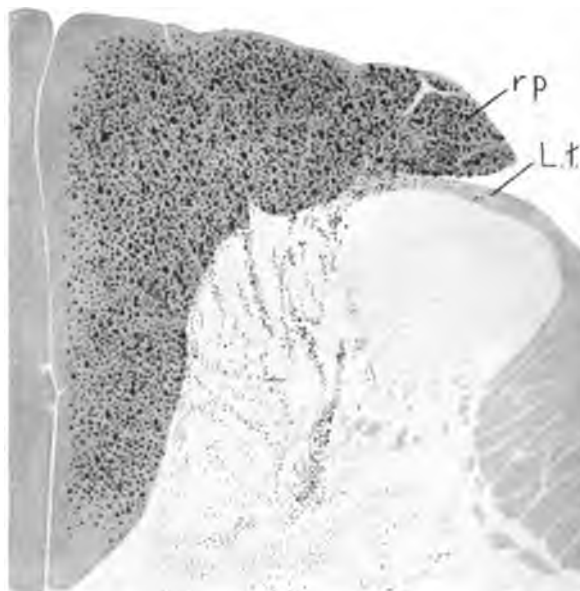


Fig. 1 From the sixth lumbar segment of the spinal cord of a cat, killed 74 days after unilateral ligation of the sixth and seventh lumbar roots. Notice the degeneration in the medial half of Lissauer's tract and in the fasciculus cuneatus. Pal-Weigert. $\times 28$. *f.c.*, fasciculus cuneatus; *s.g.R.*, substantia gelatinosa Rolandi; *s.z.*, stratum zonale; *L.t.*, Lissauer's tract; *r.p.*, radix posterior.



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Katharine Hill.

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Fig. 2 From the same section as figure 1, showing the medullated fibers in the partly degenerated tract of Lissauer. The left side of the drawing is from the lateral half of the tract and shows the normal number of medullated fibers; the right side is from the medial half of the tract and shows a marked decrease in the number of medullated fibers. Pal-Weigert. $\times 400$.

Fig. 3 From the seventh lumbar segment of the spinal cord of a cat, killed 20 days after the unilateral division of the sixth and seventh lumbar roots. Notice the degeneration in the cuneate fasciculus and in the medial half of the tract of Lissauer. Marchi. $\times 40$. *L.t.*, Lissauer's tract; *r.p.*, radix posterior.

upward into the fifth lumbar is much greater than the number which extend downward into the first sacral segment.

Both figures 1 and 2 were taken from a section at the middle of the sixth lumbar segment 74 days after the sixth and seventh roots had been destroyed, and represent the maximum amount of degeneration of medullated fibers obtainable in Lissauer's tract by destruction of the dorsal roots. We say that it is the maximum, because the time allowed for the degeneration is adequate and because, as we have seen, very few medullated root fibers ascend more than one and a half or descend more than half a segment in the tract of Lissauer. It is doubtful if destroying the fifth lumbar and first sacral roots in addition to the sixth and seventh lumbar would have caused the disappearance of any more of the fibers from the tract in the middle of the sixth lumbar segment.

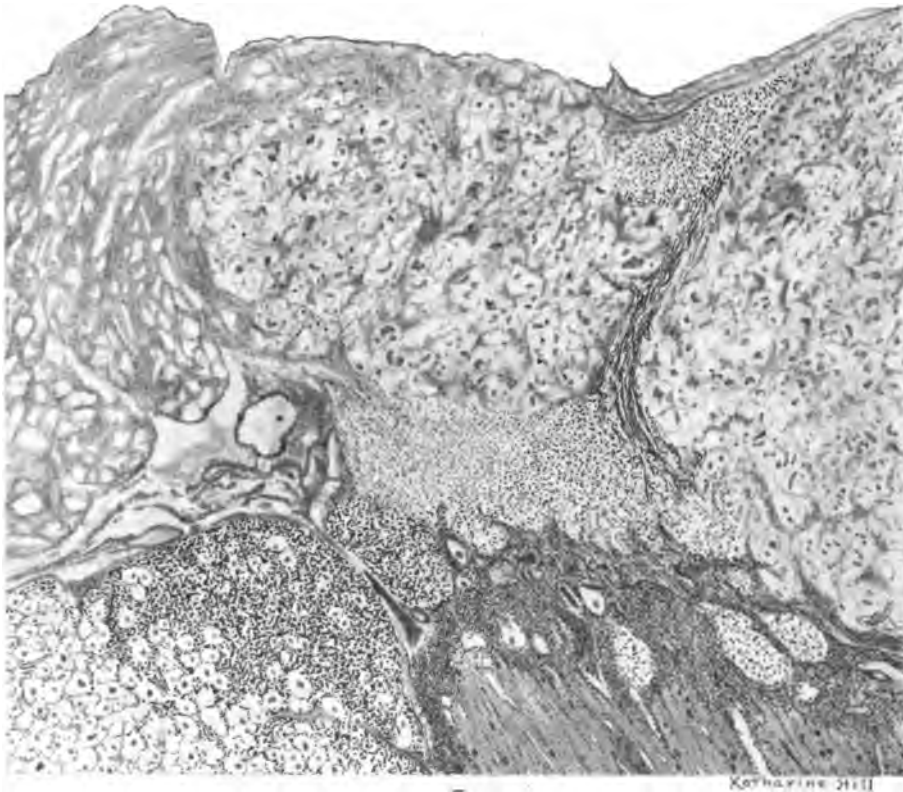
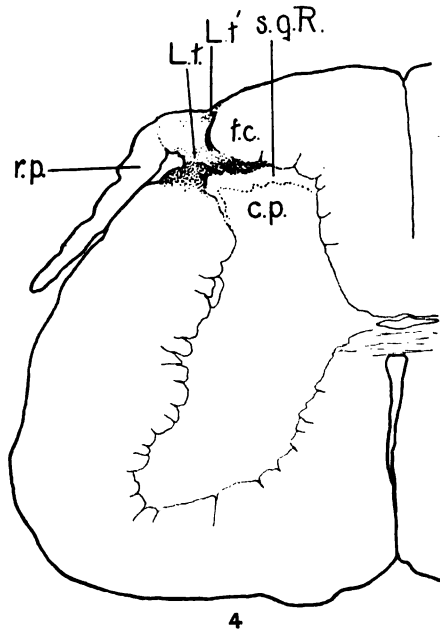
In view of these facts, it is clear that the fibers indicated in figure 2 are all or nearly all endogenous. That is to say, in the lateral half of Lissauer's tract (in the sixth lumbar segment of the spinal cord of the cat) practically all of the medullated fibers are endogenous, while in the medial half a large number, perhaps 50 per cent, are endogenous.

The degeneration of the non-medullated fibers can not be followed in so definite a way as that of the medullated fibers, since we have no stain for degenerated axons similar to the Marchi stain for degenerated myelin. It must be studied in a negative way, just as the degeneration of medullated fibers is studied in Pal-Weigert preparations; i.e., we have only the disappearance of the fibers as an evidence of degeneration. Furthermore, the change seen in pyridine-silver preparations will be due to a disappearance of both medullated and non-medullated axons, and it will be necessary, therefore, to speak of it as a degeneration of axons, with the understanding that the majority of the axons that disappear are non-medullated. Figure 5 illustrates how the degeneration of axons is shown by pyridine-silver preparations. About half of the tract of Lissauer takes the stain normally. This is the part situated lateral to the point of entrance of the

lateral part of the dorsal root. The medial part, approximately half, is stained very lightly and contains only a small part of the axons that are normally present. The illustration represents as high a degree of degeneration as was seen in any of the preparations. It was in the middle of a degeneration involving three segments, and was drawn from the fifth lumbar segment of Cat II in which the fourth, fifth, and sixth lumbar roots were ligated. The degeneration was at its maximum in the fifth lumbar segment and decreased in an upward and downward direction. There was no clear evidence of degeneration in the third lumbar segment. In the highest part of the fourth lumbar segment a slight degeneration was evident. It gradually became more pronounced as the series was followed downward through this segment. Throughout the fifth lumbar segment the degeneration was well marked, and gradually decreased as one passed downward through the series of the sixth lumbar segment. No degeneration could be seen in the seventh lumbar segment. These facts show that the non-medullated fibers are chiefly confined to the segment at which they enter the cord. They do, however, extend up and down to some extent, as is shown by the fact that the degeneration was greater in the fifth than in the fourth or sixth lumbar segments. From what was seen in Pal-Weigert and Marchi preparations it is clear that some medullated axons must have degenerated and disappeared from the third and seventh lumbar segments, but their absence can not be detected in pyridine-silver preparations. It is also probable that some few non-medullated axons have disappeared from these segments without bringing the total loss of axons to an amount appreciable by the method used. Even where the degeneration is at its

Fig. 4 Outline drawing to show the location from which figure 5 was drawn. *c.p.*, columna posterior; *s.g.R.*, substantia gelatinosa Rolandi; *f.c.*, fasciculus cuneatus; *L.t.*, Lissauer's tract; *L.t'*, isolated portion of Lissauer's tract; *r.p.*, radix posterior.

Fig. 5 From the fifth lumbar segment of the spinal cord of a cat, killed 24 days after the unilateral ligation of the fourth, fifth, and sixth lumbar roots. For orientation see figure 4. Notice the degeneration in the medial half of Lissauer's tract and in the entering root and cuneate fasciculus. Pyridine-silver. $\times 167$.



maximum there is still a large number of normal axons, a number far in excess of the number of surviving medullated fibers in the same position in corresponding Pal-Weigert preparations. This shows that some of the surviving axons in the medial half of the degenerated tract of Lissauer represent endogenous non-medullated fibers. The absence of visible degeneration in the lateral half shows that all or nearly all of the axons, non-medullated as well as medullated, in the lateral half of Lissauer's tract are endogenous.

As seen in figures 4 and 5, the bundle *L.t.*' in the dorso-lateral angle of the cuneate fasciculus, which has been separated from the rest of the tract of Lissauer by the entering root, does not undergo a complete degeneration. It degenerates to about the same extent as the medial half of the tract of Lissauer, of which it is a part. Occasionally, one sees bundles of axons running forward to join the main part of the tract, as seen in figure 5. The number of axons in this bundle in the dorso-lateral angle of the cuneate fasciculus, and the number and size of the bundles connecting it to the main part of the tract are much decreased on the operated side in the segments affected. Like the medial half of the tract, it is composed chiefly of dorsal root fibers. The normal appearance of this bundle in the dorso-lateral angle of the cuneate fasciculus of the fifth lumbar segment of the cat's cord is seen in figures 4 and 8 of a paper on Lissauer's tract in the cat (Ranson '13).

So far as the substantia gelatinosa Rolandi is concerned, there was no appreciable decrease in the number of axons in its various parts. This should not be taken to mean that the dorsal root fibers of Lissauer's tract do not enter the substantia gelatinosa, since the number of other axons there is so great that the loss of these from the dorsal root does not make an appreciable difference in the stain.

REGENERATION OF THE SPINAL NERVE ROOTS

In only one case was the distal part of the proximal stump of a divided ventral root included in the sections. In this case 24 days after the operation the ventral root near its cut end showed a great proliferation of axons similar to that seen in the distal end of the proximal stump of a divided peripheral nerve. Great numbers of fine new-formed axons occupied the interstices between the medullated fibers and in many places bundles of these new-formed axons filled the spaces formerly occupied by medullated fibers.

In Cats I and II, 24 days after the roots had been tied, one finds a moderate number of fine axons in the dorsal roots. These differed from the normal non-medullated fibers chiefly in their irregular course and grouping. Some were branched, but the end bulbs typical of regenerating fibers were not seen. These fibers were much more abundant near the cut end of the root than at the end next the cord. But some were present at the proximal end and a few could be seen in the root as it entered the cord. In Cat IV, 74 days after division of the roots, the dorsal roots were crowded with fine axons which resembled normal fibers. These were present in great numbers up to the point where neuroglia takes the place of connective tissue just before the root enters the cord and where the abrupt change in the character of the degeneration was seen. Up to this point the degenerated roots were crowded with new-formed axons, but beyond it only a few fine axons extended through the entering root into the cord. Similar observations were made in Cat VI and Cat X, respectively 70 and 51 days after division of the roots.

There can be no question but that the presence of these great numbers of fine axons in the degenerated roots can only be explained on the basis of their being regenerated fibers. But it is not easy to decide how to regard those few fine axons that extend beyond the transition point into the cord. It is possible that they are efferent fibers and it is equally possible that they are regenerated fibers. It is to be regretted that the spinal

ganglia were not removed in these experiments so as to exclude the possibility of regeneration. Only by excluding this possibility can the question of efferent dorsal root fibers be settled.

CONCLUSIONS

The results of these experiments confirm those of earlier histological studies and show that both fine medullated and non-medullated fibers from the dorsal root enter the tract of Lissauer.

The tract of Lissauer in the lumbo-sacral region of the cat's cord consists of two parts, approximately a medial and a lateral half. The fibers of the lateral half, both medullated and non-medullated, are of endogenous origin. The fibers of the medial half, both medullated and non-medullated, are in part endogenous and in part exogenous. While it may be that in other animals there would not be this sharp division of the tract of Lissauer into medial and lateral halves, we may safely generalize our conclusions so far as to say that the tract of Lissauer is a mixed tract consisting of medullated and non-medullated fibers of both endogenous and exogenous origin. Variations in the shape of the tract in different animals are probably associated with variations in the development and position of the endogenous fibers of the tract.

Our results do not permit us to trace the non-medullated fibers to their termination in the cord nor to decide the question of the existence of efferent dorsal root fibers.

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TRANSPLANTATION OF THE SPINAL GANGLION, WITH OBSERVATIONS ON THE SIGNIFICANCE OF THE COMPLEX TYPES OF SPINAL GANGLION CELLS

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FIVE FIGURES

One may no longer look upon the spinal ganglion as containing only simple unipolar cells with T-shaped axons. The researches of the past ten years have shown that from this fundamental type all sorts of variations occur. A glance at Dogiel's ('08) monograph shows how great is the number of such variations and how completely they baffle any attempt at logical classification. From the cell body may arise short, thick processes, or fine fibers ending in pyriform or spherical expansions. Other cells present elevated loops of protoplasm which are attached to the cell body at both ends. When such loops are numerous, the cells are spoken of as fenestrated. In other cases the axon at or near its origin from the cell may be broken up into a number of fibers, which unite with each other to form a plexus, and which are finally assembled into a single axon. Other axons give off collaterals with terminal enlargements called end-bulbs.

Although these complicated structures have been much discussed, we are still in doubt as to their functional significance. In this connection, we should not forget that, although obscured by all these variations, the fundamental characteristic of the spinal ganglion cell remains unchanged; i.e., the cell possesses an axon which, dividing dichotomously, puts the cell into relation with the periphery on the one hand and the central nervous system on the other, and, so far as we understand the physiology of the spinal ganglion, this simple type seems to answer all the requirements for the conduction of afferent impulses.

It is possible, of course, that these complex formations in the spinal ganglion may furnish points of contact for the transmission of nervous impulses between afferent neurones or even between afferent and sympathetic neurones. But there is no physiological evidence that such an interchange of impulses does occur in the spinal ganglion. Furthermore, these branches, bulbs, and plexuses seem to be arranged in a manner unfavorable for the occurrence of points of contact between neurones. Only in a few cases, as where the end bulb on the branch of one cell lies under the capsule of another cell, has the possibility of such contact been demonstrated.

It must also be remembered that these multiple processes, bulbs, and plexuses vary greatly in their development in different animals. In the dog the simple unipolar type represents the vast majority of the cells, while in man the more complex forms predominate. In view of this great variation in their development, it is clear that whatever function these accessory structures may have, it can not be of a fundamental character.

It was at first supposed by Cajal and others that it would be possible to classify the spinal ganglion cells into functionally separate and distinct groups according to these variations in their external form. This position is still maintained by some, but it is becoming evident that it is untenable. Two reasons for abandoning this position have already been presented; namely, the apparent ineffectiveness of these processes for transmitting nerve impulses, and the great variation in their development in closely related groups of animals.

The third reason for abandoning the conception that these alterations from the simple type represent the outward characteristics of fixed and functionally distinct groups of spinal ganglion cells is found in observations on pathological and transplanted spinal ganglia. These observations show that the form of a given spinal ganglion cell is not fixed, that it may undergo rapid changes, and that under proper stimulation cells of the simple unipolar type may be transformed into complex cells similar to those seen in normal ganglia. Such observations have been made by a number of observers.

Nageotte ('06) was the first to make observations of this sort on pathological ganglia. Studying the dorsal roots and spinal ganglia from cases of tabes dorsalis, he found in the dorsal roots many very fine non-medullated fibers, on the ends of which could be seen bulbs similar to those seen by Cajal on the tip of regenerating nerve fibers. Some of these fibers were processes from the body of the spinal ganglion cells, others were collaterals from the axons. He regarded these findings as an evidence of a collateral regeneration in contradistinction to regeneration from the end of the surviving portion of an injured axon. He believed that such a collateral regeneration was responsible for the similar structures seen in normal ganglia.

These observations on tabetic material have been confirmed by Marinesco and Minea ('07), and Bielschowsky ('08). In a case of carcinomatous metastasis, where the lesion was within the spinal ganglion, Bielschowsky found many atypical cells with multiple processes and many fine, new-formed fibers. He believes that the fenestrated cells, the cells with fine multiple branches, and the cells whose axons give off collaterals with end bulbs, seen in normal ganglia, are similar to those produced under pathological conditions, and accepts Nageotte's theory of collateral regeneration. Dejerine and André-Thomas ('07) report numerous collaterals with end bulbs in a case of herpes zoster.

In order to determine if similar changes could be produced experimentally, Nageotte ('07) transplanted spinal ganglia beneath the skin of the ear in young rabbits. Fifteen days after transplantation he found that some cells at the periphery of the transplanted ganglion had survived, but that they had taken on an appearance very different from the normal. The cell body was distorted, the nucleus excentric, and the glomerulus missing; but there were a number of processes, both fine and coarse, running in every direction from the cell. Many of these branches had bulbed extremities. There is no essential difference, according to Nageotte, between these and the similar cells found by Cajal under normal conditions. Nageotte also describes in a graft of eight days' standing: (1) cells with persistent axons from which collaterals arise; (2) cells, the bodies of which are

divided into several lobes connected by narrow necks; (3) complicated pericellular networks formed of great numbers of fine branches and collaterals; and (4) glomeruli of fine fibers occupying the capsules of dead cells, and formed by collaterals from adjacent axons.

Similar observations on transplanted ganglia have been made by Marinesco and Minea ('07-'08), and by Agosti ('11). Quite similar changes have been seen when spinal ganglia have been cultivated in vitro (Lengendre and Minot '11, and Marinesco and Minea '12, '14).

Our own observations confirm those of previous investigators that simple unipolar cells may be transformed under experimental conditions into complex multipolar cells. An additional fact of importance brought out by this investigation is that such multipolar cells can return again to their original simple form.

In this work white rats were used, two for each experiment. The animals receiving the graft varied in age from one month up. The grafts were taken from animals of varying ages, some only one week old. The head of the recipient was prepared and a small strip of bone removed parallel to the superior sagittal sinus, and an incision made in the cerebral cortex in line with the bone defect. A warm pack was then placed over the wound and the second cervical spinal ganglion removed from the neck of the second rat. The ganglion was grasped with fine forceps by a short stretch of attached nerve and inserted into the brain wound in such a way that the nerve was deepest in the wound and the ganglion just beneath the cortex.

The first experiments were made in 1905. At that time three successful transplantations were made. In each of these experiments the recipient was one month old, and the donor a week old. Two animals were allowed to live ten days and one two months. The brains from the first two animals were examined for the site of the graft and this with a piece of the surrounding cortex was excised. Paraffin sections from these were stained with toluidin blue and erythrosin. The third brain was prepared by the Pal-Weigert method and counterstained with Upson's carmine.

The sections from the brain of Rat I containing a graft of ten days' standing shows the spinal ganglion imbedded in the substantia alba just dorsal to the radiations of the corpus callosum. It is easily recognized by the abundance of connective tissue and by the presence of the round or polygonal cells characteristic of the spinal ganglion. These are arranged in the shape of a horseshoe, two or three cells deep, around the periphery of the ganglion on the side toward the corpus callosum. All nerve cells have disappeared from the interior of the ganglion. The surviving cells are in various stages of chromatolysis, but the majority of the cells show only partial solution of the tigroid masses. These cells are much swollen and their nuclei are excentric. No small nerve cells can be seen. These are more susceptible to injury than the larger cells and rapidly disappear from the transplanted ganglia.

Substantially the same conditions are to be seen in Rat II, also representing a ten-day graft.

The spinal ganglion which was allowed to remain in the brain for two months is shown in figure 1. The graft was not as deeply placed as the others, but was imbedded in the cortex near the great longitudinal fissure and covered over by a proliferation of the pia mater. The ganglion is much decreased in size and surrounded by scar tissue. It contains only a small fraction of the number of cells to be found in a normal ganglion, but these few cells are of normal appearance, so far as can be ascertained by the carmine stain. The shape and size of the cell body, the position, size and contour of the nucleus and the absence of any proliferation of the nuclei of their capsules justify one in assuming that these cells are not undergoing degeneration but have survived the transplantation and would continue to exist indefinitely in their new position.

A few medullated nerve fibers can be seen in the ganglion. These are gathered together in a bundle which can be traced for some distance in the scar when a series of these sections are studied. It is not possible to say whether these are regenerated fibers or fibers which have persisted from the time of transplantation of the ganglion.

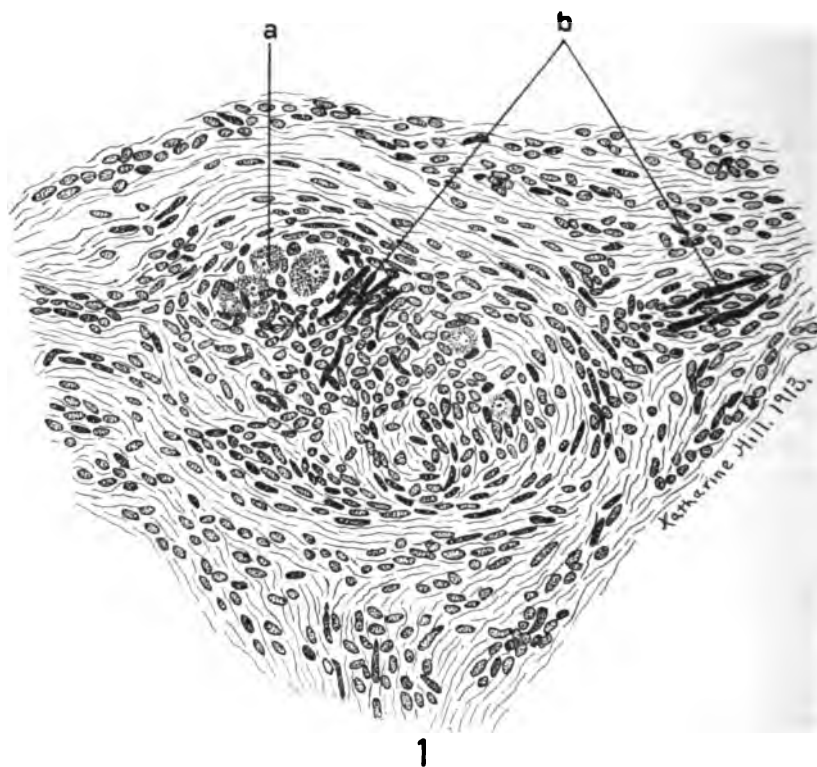


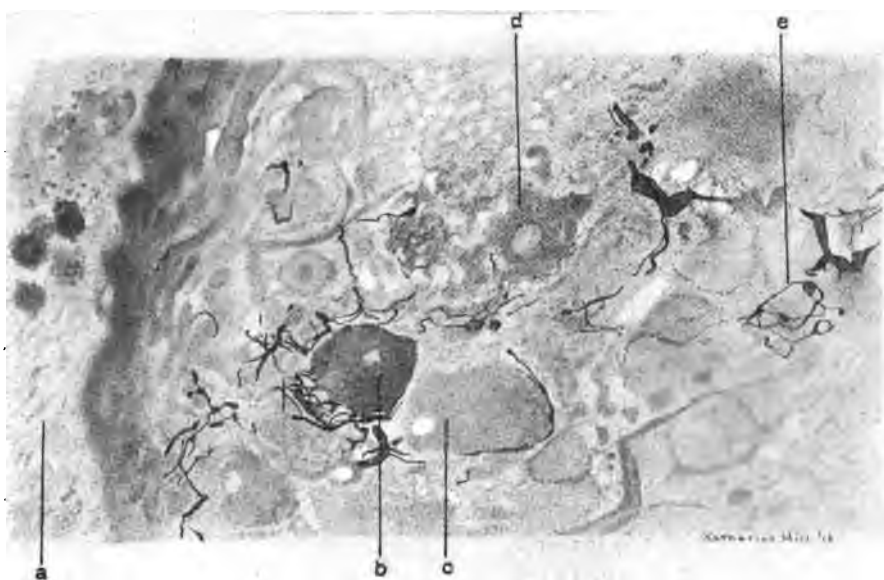
Fig. 1 Transplanted spinal ganglion of the rat; duration two months. Shows several spinal ganglion cells, *a*, and a bundle of medullated nerve fibers. *b*. Pal-Weigert and Upson's carmine. $\times 180$.

Fig. 2 Transplanted spinal ganglion of the rat; duration three days. *a*, coagulum surrounding the ganglion; *b*, living cell surrounded by new-formed fibers; *c* and *d*, disintegrating cells; *e*, plexus of new-formed fibers. Pyridine-silver. $\times 544$.

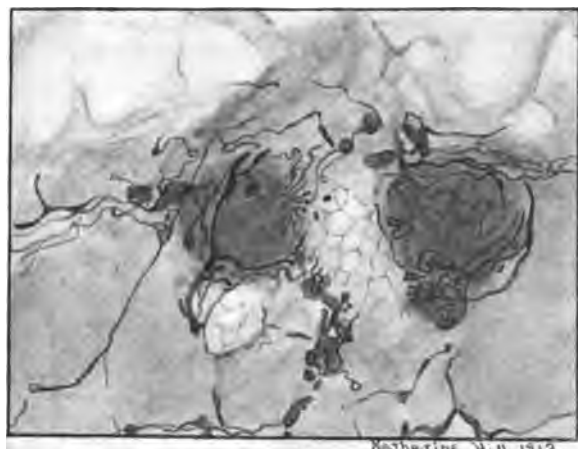
Fig. 3 Cells from a transplanted spinal ganglion of the rat; duration four days. Pyridine-silver. $\times 544$.

Fig. 4 Cell from a transplanted spinal ganglion of the rat; duration two days. Pyridine-silver. $\times 544$.

Fig. 5 Transplanted spinal ganglion of the rat; duration 17 days. Pyridine-silver. $\times 352$.



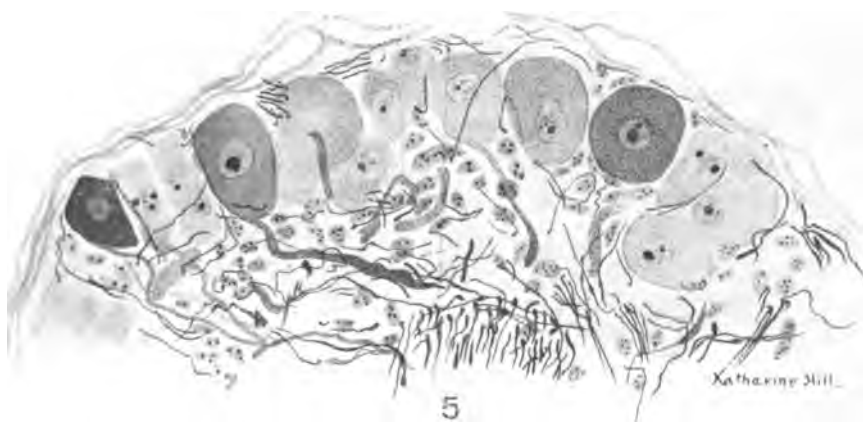
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In a second series of experiments six successful transplantations were made. These fall into two groups according to the period of survival. In the first group of three, one rat was allowed to live two days, one three, and one four days. In the second group of three, one was killed on the 13th, one on the 17th, and one on the 18th day. In these six experiments all the animals used were half grown or young adults.

Our observations on the first group confirm those of others on transplanted ganglia. The ganglion lies imbedded in the brain surrounded by a small amount of clot (fig. 2, *a*). All of the cells on the interior of the ganglion are dead. A few cells near the periphery of the ganglion have survived. These living cells stain much darker than the dead ones and sometimes show a clear neurofibrillar network. They are always surrounded by a wealth of new-formed fibers (fig. 2, *b*). Some cells seem to have undergone a temporary reaction and then succumbed (fig. 2, *d*). Everywhere in the neighborhood of reacting cells, branching fibers and plexuses (fig. 2, *e*) are to be seen. In most of these reacting cells it is not possible to distinguish the original axon, which has probably either entirely disappeared or broken up into fine branches. The cell is surrounded by a plexus of branching fibers, irregular in their contour, with swellings in their course, and often ending in bulbs or rings (figs. 3 and 4). The fine fibers may arise either directly from the cell body or from the coarse branches, and the fibers often unite in a true plexus formation. These new-formed fibers closely resemble those seen in the early stage of regeneration of a peripheral nerve.

The grafts of 13, 17, and 18 days' standing presented a very different picture, since the living cells in them had returned to a condition approaching the normal. This restoration was probably rendered possible by several favorable factors in the technique. In the first place, the second cervical ganglion of the rat is a very small piece of tissue, through which nutrient fluids can penetrate easily. It is readily isolated and handled without injury to itself by grasping the attached nerve with fine tissue forceps. No sutures were needed to hold it in place in the brain, and it seems probable that the brain may be a more favorable

site for the transplantation of nervous tissue than is subcutaneous tissue or muscle.

After two weeks most of the bizarre formations have disappeared from the transplanted ganglion. As seen in figure 5, which represents a graft of 17 days' standing, most of the dead cells have been removed; the ganglion has shrunk; and the living cells have been brought close together near the periphery of the ganglion. These cells have a smooth well-defined contour and have a single axon, running toward the center of the ganglion and showing practically no glomerulus. The multiple fine and coarse branches of the cells have disappeared, but there are numerous fine, even-contoured fibers running through the ganglion in every direction. Only in a small proportion of the cells could any process be demonstrated and this, when present, appeared to be a typical axon. In one section (fig. 5) a number of the cells showed axons running toward the center of the ganglion.

In each of the three grafts of two or three days' duration the surviving cells were multipolar and in each of the three of 13 to 18 days' duration the cells had returned to their normal form. The essential point in these results is that, under suitable experimental conditions, unipolar cells may transform themselves into multipolar cells and later return again to their original form.

The spinal ganglion cells do not undergo permanent changes in form after division of their axons at a distant point in a peripheral nerve. A month after division of the sciatic nerve in dogs the cells in the associated spinal ganglia were of normal form. Nor are such changes produced by cutting the dorsal root a short distance above the ganglion. In connection with a study of Lissauer's tract I have divided the dorsal roots of the sixth and seventh lumbar and first sacral nerves proximal to the ganglia in a number of cats, and have taken advantage of the opportunity to study the ganglia associated with the cut roots in four cats which had survived the operation, respectively, 24, 70, 74 and 74 days. On comparing these with the normal ganglia, no change in the external form of the cell could be observed. While these experiments show that division of the axon at a distance does not produce permanent changes in the external form of the spinal

ganglion cells, they do not exclude the possibility that transitory changes may occur during the first week or two after the lesion.

This question of the variation in external form of the spinal ganglion cells needs further study. Levi ('07) and Huber ('13) have shown that cells with fine processes ending in bulbs are to be found in the spinal ganglion during late foetal life and shortly after birth. The early appearance of these structures is an important fact to bear in mind in estimating their significance, and evidently points in the opposite direction from the evidence presented in this paper. But this much is clear, that under pathological conditions and in transplanted ganglia simple unipolar spinal ganglion cells become transformed into complex multipolar cells, due to the sprouting of new processes from the cell body and axon, and that these complex cells are very similar to, if not identical with, those found in varying numbers in normal ganglia. It is also clear that these new-formed processes can later disappear and the cell again be transformed into the simple unipolar type. In this way it is demonstrated that the form of the spinal ganglion cell is not stable and fixed, but is capable of undergoing marked alteration in a short space of time. It is probable that the similar complex cell types seen in normal ganglia in such varying numbers are not characteristic of this or that functionally distinct group of neurones, but rather a transient expression of the physiological condition of the neurone.

Just what the factors are which bring about such changes in form is here left unmentioned because we have no adequate means for forming a conception of them, and what theorizing has been done on the subject has only tended to obscure the essential fact that the spinal ganglion cells readily undergo striking changes in form.

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